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Exposure Assessment for *Mycobacterium avium* subspecies *paratuberculosis*

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Glossary

MAP: *Mycobacterium avium* subspecies *paratuberculosis*

Animal types

Adult sheep: Sheep older than 2 years of age.

Young sheep: Sheep of approximately 12 months of age.

Adult dairy cows: Cows from dairy herds older than 2 years of age, approximately 400 to 500 kg live weight.

Young beef cattle: Animals with less than 2 years of age within the Yearling steer (330 to 400 kg live weight; domestic market) and Medium steer (400 to 500 kg live weight; export market) categories according to MLA Market Information Services.

Infection status

Infected flock or herd: A flock or herd with one or more animals infected with *Mycobacterium avium* subspecies *paratuberculosis*. This term as used in the report includes known infected flocks/herds and ones that are infected but this status has not been identified and designated.

Disease pathology type

Sheep:

- No MAP: Non-infected animals and animals with disease pathology lesions score Perez 1 and 2
- Low MAP level: Animals with paucibacillary lesions
- High MAP level: Animals with multibacillary lesions

Cattle:

- No MAP: Non-infected animals and animals with no lesions, focal and multifocal lesions
- Low MAP level: Animals with paucibacillary lesions
- High MAP level: Animals with multibacillary lesions

Meat type

Prime cuts: whole muscle

Boning room trim: skeletal muscle

Johne's disease prevalence areas for cattle

Free zones: Free zones are areas where Johne's disease is not known to exist. The only Free zone is Western Australia at this stage.
Protected Zones: Protected zones are those areas where there is little or no evidence of Johne's disease.
Beef Protected Area: The new Beef Protected Area covers all of New South Wales and the southern agricultural regions of South Australia. This area is being established because Bovine Johne's disease among beef herds in the present Control Zones is rare. Because of the much higher incidence of infected herds in the dairy population, it aims to separate beef and dairy enterprises so that the different levels of risk can be managed
Management Area: The Management Area covers all of Victoria and mainland Tasmania, where the disease is well established in the dairy industry and where there is a voluntary approach to limiting its spread.

Abstract

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's Disease in cattle, sheep and other ruminant species. Growing speculation about the role of MAP in the pathogenesis of Crohn's Disease has provoked interest in reducing the exposure of humans to MAP. The aim of this project was to assess the likelihood of exposure of humans to MAP through the consumption of red meat and to identify possible mitigation strategies to minimize this exposure. This quantitative exposure assessment identified the potential pathways of exposure, focusing on direct consignments from beef and dairy cattle and sheep properties and estimated the amount of MAP present in muscle, liver and intestines at the end of the abattoir slaughter line. A combination of peer-reviewed literature, industry reports, unpublished studies and expert opinion were used to populate the models. Results from these assessments indicate that most animals entering the slaughter line are non-infected animals from non-infected flocks/herds. Of animals coming from infected flocks/herds, most animals are non-infected with only low level MAP on the carcass surface due to cross-contamination; with an extremely low proportion of infected animals with high level of MAP present on the carcass surface. Adult sheep from high prevalence areas and dairy cattle from management areas are the animals posing the highest probability of exposure. The most influential parameters on the probability of exposure to and the amount of MAP present in product are (1) whether sheep flocks are vaccinated against Johne's disease and (2) the quantity of faeces present on the carcass surface of sheep and cattle. The study suggests that it is possible for MAP to be present in red meat products. However; the risk of human exposure through the red meat chain in Australia is low.

Executive summary

Mycobacterium avium subspecies *paratuberculosis* (MAP) causes Johne's Disease in cattle sheep and other ruminant species. The link between exposure to MAP and the development of Crohn's disease in humans is not conclusive but speculation about it is increasing. This has already started to impact the animal and food industries. For this reason it is conceivable that /pressure will at some point be placed on the red meat industry in Australia to demonstrate the measures it is taking or could take to reduce the possibility of exposure. The general objective of this project was to assess the likelihood of exposure of humans to MAP through the consumption of red meat and to identify possible mitigation strategies to minimize this exposure in Australia.

This assessment identified the potential pathways of exposure of humans to MAP via red meat focusing on the beef and dairy cattle and sheep industries by estimating the probability of this exposure to occur and the amount of MAP in product at the end of the slaughter line. The exposure assessment followed the World Organization for Animal Health methodology for risk analysis (OIE, 2009) and used a modular process risk model approach to develop the scenario trees representing the potential pathways of exposure. The modules considered were on-farm, lairage at the abattoir and the abattoir slaughter floor. The amount of MAP present in the different types of products was estimated using a simulation model representing batches of animals going through the abattoir. Quantitative stochastic simulation modelling was used to obtain outputs of the model and a sensitivity analysis was conducted to identify those input parameters with more influence on the outputs. A combination of peer-reviewed literature, industry reports, unpublished studies and expert opinion were used to define the pathways and parameterized the input values required to populate the models.

Different scenarios were used to compare the risk of MAP exposure posed by animals originating in different regions or geographical areas of Australia, with differing prevalences of Johne's disease, and from different production types (e.g. beef vs. dairy). Each of these scenarios provides an independent outcome on the potential risk of exposure, allowing for comparison between them. For sheep, three geographical areas were considered, depending on the Johne's disease flock prevalence (GA1[1], Extremely low prevalence; GA2, High prevalence; GA3, Low prevalence). Within each of these geographical areas, two types of consignments sent to the abattoir were considered: Adult sheep, described as those animals older than 2 years of age; and, young sheep, described as those animals of

[1] GA1 e.g. Western New South Wales (NSW), Northern South Australia; GA2, e.g. Southern Tablelands NSW; GA3, e.g. Northern Tablelands NSW, South-Eastern South Australia

approximately 12 months of age. For cattle, the scenarios considered also depended on the geographical area, as per the Bovine Johne's disease control program map, and the type of animals sent to the abattoir (S1[2], Beef, in Protected and Free zones; S2, Beef, in Beef Protected and Management areas; S3, Dairy in Beef Protected areas; S4, Dairy in Management area; S5, Dairy in Protected zone). The type of consignments considered for this assessment were: Adult dairy cows (> 2 years old, 400 to 500 kg live weight, domestic market); and, young beef cattle (< 2 years old, 330 to 500 kg live weight; domestic and export market). The products considered were: whole muscle or prime cuts, intestines, liver and skeletal muscle. For each consignment entering the slaughter line the probability of non-infected and infected animals was estimated. Among infected animals, the probability of animals being infected with low or high level of MAP and with low or high MAP contamination on the carcass surface, were also estimated. In addition, the amount of MAP in a serving size of the product obtained among all animals within an infected consignment was estimated.

Results of the sheep assessment indicate that most animals from GA1 and GA3 (>95%) entering the slaughter line are estimated to be non-infected animals from non-infected flocks, posing no risk of MAP exposure to humans. Among consignments originating from GA2, a lower proportion (70.0%) is estimated to be non-infected animals from non-infected flocks, due to the higher flock prevalence in this area, posing a higher overall risk of exposure to MAP to humans than consignments from GA1 and GA3. Of the remaining animals, most would have low levels of MAP on the carcass surface due to cross-contamination in lairage but not be infected. An extremely low to negligible proportion of animals would be infected and have high level of MAP contamination on the carcass surface. The amount of MAP in final product for an average animal within an infected consignment ranged from 0.45 to 1.24 Log (Log10) CFU in 100g of skeletal or whole muscle and up to 7.30 logs CFU in 10g of intestines

Results of the cattle assessment indicate that most animals (>99%) from S1, S2 and S5 will be non-infected animals from non-infected herds, and will not pose a risk of MAP exposure to humans. This proportion is lower for dairy cattle consignments from S3 (88.4%) and S4 (79.8%), as the herd prevalence in these areas is higher. Similar to sheep, of the remaining animals from all the scenarios considered in this assessment, most cattle would be non-infected with low level of MAP contamination on the carcass surface, and an extremely low

[2] S1, e.g. Western Australia; Queensland; Northern Territory, pastoral zone South Australia, Flinders Island; S2, e.g. New South Wales; South-Eastern South Australia; S3, e.g. New South Wales; South-Eastern South Australia; S4, e.g. Victoria, Tasmania; S5, e.g. Queensland; Northern Territory, pastoral zone South Australia, Flinders Island.

to negligible proportion would be infected animals with high level of MAP contamination. The amount of MAP in product is estimated to be lower than for sheep, with a median -0.02 MAP Log CFU for 100g of prime cut from beef cattle to 0.16 MAP Log CFU for 100g of skeletal muscle from dairy cows.

The sensitivity analysis indicated that the most influential parameters on the probability of exposure to and the amount of MAP present in product are (1) whether sheep flocks are vaccinated against Johne's disease and (2) the quantity of faeces present on the carcass surface of sheep and cattle.

Results from this study, provide an insight into the risk of exposure of humans to MAP posed by the consumption of red meat from the cattle, dairy and sheep industries in Australia, and identify which geographical areas and production systems pose a comparatively higher risk. Results suggest that the risk posed by the red meat chain in Australia is low as most animals entering the slaughter line destined for human consumption originate from non-infected properties. However, these results also suggest that the risk is not negligible, and that measures to reduce the flock and herd prevalence as well as the animal prevalence, and to maintain good hygienic practices at the abattoirs to avoid or reduce carcass contamination are crucial to control and manage this risk. This study has also highlighted the need for further research that will enable better quantification of the risk to humans from MAP. Specific topics for further research include, disease prevalence, disease pathology, the amount of MAP on carcases and in the end products consumed

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1 Introduction

Johne's disease (paratuberculosis) is a chronic contagious granulomatous enteritis of domestic and wild ruminants. It is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), a hardy, slow-growing, gram-positive, and acid-fast bacterium (Tiwari et al., 2006). Paratuberculosis is a multiple species disease listed by the World Organization for Animal Health (OIE) Terrestrial Animal Health Code. The natural hosts for MAP are wild and domesticated ruminants, including dairy and beef cattle, sheep, goats, cervids and camelids. The route of infection is usually through ingestion via contaminated water, milk, or feed(Tiwari et al., 2006). Susceptibility to infection is highest in newborn animals and decreases with age(Windsor and Whittington, 2010). The course of the disease has been divided into different stages. Sheep typically develop lesions within 6-12 months following detection of infection by culture (Dennis et al., 2011). In cattle the detection of infection is difficult until cows enter subclinical stage 2 after 2-5 years, during which shedding occurs intermittently (van Roermund et al., 2007). MAP is excreted in large numbers in faeces of infected animals and it is resistant to environmental factors and can survive on pasture for >1 year; survival in water is longer than in soil. The infection is usually acquired through the faecal-oral route; the dose needed to infect an animal is not known. MAP can become widely distributed within the tissues of infected animals, and meat may be a possible route of exposure of MAP to humans.

An overview of recent prevalence data worldwide in beef and dairy cattle estimated that the individual prevalence varied between 0.4 and 17.5%, and herd prevalence varied between 2.6 and 70.2%, respectively (Eltholth et al., 2009). MAP was first diagnosed in cattle in Australia in 1959 and is endemic in Victoria, New South Wales, South Australia and Tasmania.

The link between exposure to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) and the development of Crohn's disease in humans is not conclusive but speculation about it is increasing. A large number of experimental and observational studies have investigated this issue. MAP has been isolated from many patients with Crohn's disease lesions (Chiodini et al., 1984; McFadden et al., 1987; Chiodini, 1989, 1990) and identified from a significantly higher proportion of Crohn's disease tissues than from controls by improved culture techniques, PCR-assays (Dell'Isola et al., 1994) and *in situ* hybridization techniques (Hulten et al., 2000b). Moreover, a MAP-specific humoral immune response was identified and macrolide and anti-mycobacterial drug therapies were efficacious in Crohn's disease patients (Naser et al., 1999; Hulten et al., 2000a) supporting the role of MAP as a causative organism of Crohn's disease. The evidence in support of MAP as a cause of Crohn's

disease is growing (Naser et al., 2000; Schwartz et al., 2000), but the scientific community is divided over its role. For example, in 2003 Greenstein presented the 'personal view' that MAP fulfils Koch's postulates for Crohn's disease even more than *M. leprae* fulfils these for leprosy (Greenstein, 2003) while Freeman and Noble (2005) did not find any evidence of the association of MAP with Crohn's disease.

Even though the association of MAP with Crohn's disease is yet to be confirmed, it has already started to impact the animal and food industries. Public health authorities have started to prepare themselves to meet this challenge. The possibility of transmission of MAP from infected cattle to humans via contaminated milk or meat is being investigated in many countries. For example, in Switzerland, 9.7% of bulk-milk samples were found to be *IS900* PCR-positive, indicating that MAP could be transmitted to humans by raw milk consumption (Corti and Stephan, 2002). Similar findings were reported from studies in the Czech Republic (Ayele et al., 2005) and the UK (Grant, 2003). MAP has been shown to be capable of surviving milk pasteurisation in the UK and USA (Grant, 2003; Stabel and Lambertz, 2004). On the other hand, Holsinger et al. (1997) believed that the current standards for public health assurance of milk safety were adequate, provided good manufacturing practices were followed. Similarly, a study conducted in Australia determined that pasteurization was very effective in killing MAP (McDonald et al., 2005).

Similarly, there have been conflicting reports of the presence of MAP in meat. In the United States while Jaravata et al. (2007) could not detect presence of MAP in retail ground beef, Meadus et al. (2008) did detect MAP genome in two swab samples from the anal region of 450 skinned and dressed beef carcasses taken after carcass pasteurisation with hot water or steam, but concluded that the surface carcass contamination was most likely derived from the environment rather than the animal. From investigations of tissue samples using modified techniques to enhance sensitivity, there is evidence that MAP may be present in meat from infected animals at low numbers. Studies have demonstrated this for clinically affected cattle (Alonso-Hearn et al., 2009; Reddacliff et al., 2010; Pribylova et al., 2011) and sheep (Reddacliff et al., 2010; Smith et al., 2011). For subclinically infected animals, MAP DNA has also been detected in muscle samples, such as 4.5% of muscle samples from subclinical sheep (Reddacliff et al., 2010), demonstrating that dissemination does occur in these animals (Pribylova et al., 2011). Thus, although often animals for meat production are young and unlikely to be heavily infected and meat is usually consumed cooked, the presence of MAP in muscles of MAP-infected cattle destined for human consumption poses a potential risk of exposure to humans.

While considerable research on the efficacy of thermal inactivation of MAP in milk has been conducted, there are limited studies on the efficacy of food processing on the viability of

MAP in meat products (Eltholth et al., 2009; Whittington et al., 2010). In a recent study, the inactivation of MAP in lamb skeletal muscle at different temperatures was investigated (Whittington et al., 2010). At temperatures of 65–70 °C, MAP was reported to be less heat tolerant in muscle than in milk. The total thermal exposure of MAP during baking of leg-of-lamb roasts in domestic ovens was determined to result in more than 20 log reductions in most cases, resulting in a microbiologically safe product. Another recent study in Canada suggests that some MAP will survive cooking of meat to a medium rare condition (63^{o}C), but their numbers will be greatly reduced; and that cooking to a well done condition (71^{o}C) can be expected to render meat free of viable MAP (Mutharia et al., 2010). Thus research to date indicates a low probability of MAP survival provided that red meat is cooked to recommended standards.

Although not conclusive, evidence for the link between exposure to MAP and the development of Crohn's disease is highly visible to the Australian community and to our trading partners. For this reason it is conceivable that pressure will at some point be placed on industry to demonstrate the measures it is taking (or could take) to reduce the possibility of exposure.

Growing speculation about the role of MAP in the pathogenesis of Crohn's Disease has provoked interest in minimising the exposure of humans to MAP. In the future it is possible that prescribed steps to reduce human exposure to MAP may become a commercial or trade requirement that the Australian red meat industry must satisfy. It is worth noting here that scientific proof of the causal association between MAP and Crohn's disease is not essential for this to happen as public perceptions are not always based on scientific evidence. Consumer pressure may force industry bodies to prove absence of MAP from the food chain even in the absence of scientific evidence.

2 Project objectives

The general objective of this project is to assess the likelihood of exposure of humans to MAP through the consumption of red meat and to identify possible mitigation strategies to minimize human exposure along the red meat production chain in Australia.

The specific project objectives are to:

- Identify the prevalence of Johne's disease in susceptible ruminant species and the different production types in Australia.
- Identify all pathways relevant to the chain of events between distribution of Johne's affected animals through the supply chain and the exposure of consumers to MAP in red meat.
- Identify possible mitigation strategies (i.e. the type of strategy and where it will be implemented along the supply chain) to reduce human exposure to MAP along the entire red meat production chain.
- Identify areas of future research to address any current gaps.

3 Material and Methods

3.1 Project scoping

The scope of the project, including the exposure assessment framework and methodology was discussed and established through consultation with the project steering group (Appendix 1) and the MLA project managers and coordinators including four face-to-face meetings and one teleconference.

This assessment identified the potential pathways of exposure of humans to MAP through the consumption of red meat and focuses on the beef and dairy cattle and sheep industries in Australia. Different scenarios according to the geographical location, JOHNE'S DISEASE herd or flock prevalence, and type of animal being sent to the abattoir were considered. The products considered in this assessment were: Whole muscle or prime cuts, intestines, liver and skeletal muscle (boning room trim).

3.2 Exposure assessment methodology

The exposure assessment followed the World Organization for Animal Health methodology for risk analysis (OIE, 2009) and the generic framework for risk assessment and management of the Australian and New Zealand Standards for Risk Management (*AS/NZS ISO 31000:2009 — Risk Management — Principles and guidelines*). The Hazard Analysis Critical Control Point (HACCP) principles were used to describe pathways of exposure and identify potential mitigation strategies. This project conducted two independent exposure assessments, one for cattle and the other for sheep.

Scenario trees were developed with a modular process risk model (MPRM) approach to represent the potential pathways of exposure of humans to MAP considering the different steps of the red meat chain, including on-farm, abattoir lairage and abattoir slaughter floor. The MPRM is based on a quantitative microbial risk assessment (QMRA) (Nauta, 2001) and allows a pathway or market chain to be divided into processing steps for investigation of the risk of product contamination in processing stages (Nauta, 2008). Microbial risk assessment on products such as milk (Clough et al., 2009), ground beef (Cassin et al., 1998) and broiler meat (Nauta et al., 2007) have utilised this method. This approach allows mitigation measures to be evaluated at the module at which the mitigation could be implemented.

The exposure scenario trees were implemented in Microsoft Excel and probabilities of exposure were calculated with stochastic simulation modelling using the software @RISK 6.0 (Palisade Corporation, USA). Probability distributions around the input values were added to account for uncertainty in the estimates. The simulation comprised 5,000 iterations sampled using the Latin hypercube method with a fixed random seed of one.
A sensitivity analysis was conducted using the @Risk Advanced Sensitivity Analysis to identify those input parameters with more influence on the probability of exposure, allowing for the identification of those critical points of the production chain where measure could be applied to reduce this probability.

3.2.1 Definition of potential exposure pathways and scenarios considered

The process of identifying the potential pathways of exposure of humans to MAP through the consumption of red meat started with the description of the main steps in the food production chain, from the farm to the meat product obtained. Each step was described as a separate module in the overall chain and all potential pathways leading to MAP exposure to humans were represented using scenario trees. The literature review and extensive consultation with the steering group supported the description of the potential pathways of exposure and development of the scenario trees.
Initially, the following modules within the meat production chain were identified: On-farm, saleyard, transport, lairage and slaughter floor. Due to paucity of quantitative data on the impact of transport on cross-contamination and transfer of MAP between animals and following the steering group advice, the transport module was excluded. Further, after consideration of the variable geographic range of source farms for animals entering saleyards and the lack of quantitative data to inform estimation of cross-contamination at saleyards and the MAP status of saleyard consignments sent direct to abattoir, in consultation with the steering group the saleyard module was also excluded.
Different scenarios were used to compare the risk of MAP exposure posed by animals originating in different regions or geographical areas of Australia, which have different Johne's disease prevalences, and from different production types (e.g. beef vs. dairy). Each of these scenarios provides an independent outcome on the potential risk of exposure, allowing for comparison between them.
For sheep (Table 1), the scenarios considered are dependent on the geographical area from which the animals originate. Within each of these scenarios, two types of animals sent to the abattoir were considered: Adult sheep, described as those animals older than 2 years of age; and, young sheep, described as those animals of approximately 12 months of age.

After extensive consultation with the project steering group, it was decided that focusing on younger animals (e.g. 5 month old prime lamb) was not appropriate since the probability of these animals being infected and shedding MAP in faeces was extremely low, therefore the exposure risk posed by these animals would be close to negligible. Table 1 describes the type of products considered for each type of animal sent to the abattoir.

Table 1. Scenarios considered for the MAP exposure assessment to humans for meat and meat products produced from sheep.

Sheep Scenarios	**Type of animal sent to the abattoir**		**Description**	**Flock prevalence**	**Example**
	Young animals (1 y old)	Adult animals (> 2 y old)			
Geographical Area 1		Skeletal muscle (Trim)	Arid and semi-arid pastoral zone	Extremely low	Western NSW; Northern South Australia
Geographical Area 2	Whole muscle (Leg of lamb)	Skeletal muscle (Trim) Intestines	Temperate	High	Southern Tablelands NSW
Geographical Area 3	Whole muscle (Leg of lamb)	Skeletal muscle (Trim) Intestines	Temperate	Low	Northern Tablelands NSW; South-Eastern South Australia

For cattle (Table 2), the scenarios considered are also dependent on the geographical area, as per the Bovine Johne's disease control program map, the type of animals sent to the abattoir and the type of product. The type of animals considered for this assessment are: Adult dairy cows, described as those animals from dairy herds older than 2 years of age, approximately 400 to 500 kg live weight; and, young beef cattle, described as those animals with less than 2 years of age within the Yearling steer (330 to 400 kg live weight; domestic market) and Medium steer (400 to 500 kg live weight; export market) categories according to MLA Market Information Services.

For each of the above listed sheep and cattle scenarios considered in this assessment, and for each module (on-farm, lairage, slaughter floor) within each subsector, a scenario tree was developed and implemented to calculate the probability of exposure. This process involved:

1) Developing the diagrams representing the exposure pathways in each module and for each animal species;
2) Identifying and describing the nodes and corresponding branches for the scenario trees representing each module;
3) Building the framework for the risk assessment model for each of the modules, allowing for the calculation of the outcome probabilities once the input values were incorporated; and

4) Linking all the modules to obtain the overall probability of exposure for each specific animal species, type of production and geographical area and using the outcome of one module as the input for the subsequent module (e.g. the on-farm outcome is the input for the lairage module).

Table 2. Scenarios considered for the MAP exposure assessment to humans for meat and meat products derived from cattle.

Cattle Scenarios	**Type of animals sent to the abattoir**		**Description**	**Herd prevalence**	**Example**
	Young cattle (< 2y old)	Adult cattle (> 2 y old)			
Scenario 1	Prime cut		Beef cattle from Protected and Free zones	Negligible	Western Australia; Queensland; Northern Territory, pastoral zone South Australia, Flinders Island
Scenario 2	Prime cut Liver		Beef cattle from Beef Protected and Management areas	Very low / Low	New South Wales; South-Eastern South Australia
Scenario 3		Skeletal muscle (trim) Intestines	Dairy properties from Beef Protected areas	Moderate	New South Wales; South-Eastern South Australia
Scenario 4		Skeletal muscle (trim) Intestines	Dairy properties from Management area	High	Victoria; Tasmania
Scenario 5		Skeletal muscle (trim) Intestines	Dairy properties from Protected zone	Low	Queensland; Northern Territory, pastoral zone South Australia, Flinders Island

3.2.2 Simulation model to estimate the amount of MAP present in product

The amount of MAP (Log10) present in the different types of products considered in this assessment and for each type of consignment arriving at the abattoir was estimated using simulated batches of animals going through the abattoir. Each batch of animals had 50 individuals, a proportion of which would be infected with high or low levels of MAP, depending on the outputs of the lairage scenario trees. The outcome from this simulation is the average amount of MAP in a serving size of the product considered among all animals in the consignment. The input parameters required for this simulation are described later in this report.

3.3 Data sources

Input parameters to populate the exposure assessments were obtained from the following data gathering activities, except where other sources are specified.

3.3.1 Literature review

A systematic review of available literature and industry statistics was conducted, which addressed the following main aspects:

1. Overview of the Australia's livestock industries with emphasis on red meat industries
2. The current status of Johne's disease in Australia, including epidemiology, prevalence and disease management
3. Likelihood of MAP exposure to humans through the red meat chain

The core of the review of key-references was a combined PubMed, Scopus and Web of Science literature search. For each of the three major topics a combined search string for the organism and keywords (depending on the topic) was carried out with limitation for publication date within the last 15 years (or older if an important key reference). In addition, available industry statistics were also reviewed; data on current status of Johne's disease in Australia was made available to the research team by Animal Health Australia through the National Johne's Disease Control Program.

The final version of the literature review was submitted to MLA in December 2012.

3.3.2 Expert consultation

3.3.2.1 Project Steering Group:

On-going consultation with the Project Steering group was conducted from the start of the project to support the development of the pathways of exposure and to obtain information to estimate the required input values to populate the exposure assessment model. Other experts consulted were: Dr Ian Links, (former National Surveillance Coordinator with the National Sheep Health Monitoring Program), Dr Rob Barwell (NSW DPI), Drs Bruce Jackson and Rowena Bell (Tasmania DPIPWE) (state coordinators of the Ovine Johne's disease control program).

3.3.2.2 Expert elicitation exercises:

A formal expert consultation was conducted to support estimation of input values required for the model, following identification of the information gaps. These exercises were conducted to provide input values in relation to the following parameters:

a. Sheep flock vaccination against JOHNE'S DISEASE
b. Disease pathology in sheep and cattle
c. Antemortem inspection and pre-slaughter practices and activities at the abattoir
d. Grams of faeces present in carcasses at the end of the slaughter line

Elicitation of expert opinion is important for risk assessment when appropriate data are limited or not available. The current assessment uses a 4-step interval elicitation process (**Figure 1**) as described by Speirs-Bridge et al. (2010), which is based on a Delphi approach (Vose, 2008). During the 4-step interval elicitation process experts are asked for a lower limit, upper limit and best guess for the estimate and their expected confidence in the interval produced during. From the first round of answers, the corresponding 80% intervals (derived interval), representing the intervals with an 80% chance of including the true value, are then obtained to compare intervals among experts and to be presented to participants. Experts are then asked to review their answers considering to the first round of responses. In the current assessment, using a second round of answers was only used to elicit the grams of faeces present in carcasses at the end of the slaughter line, due to logistic constraints.

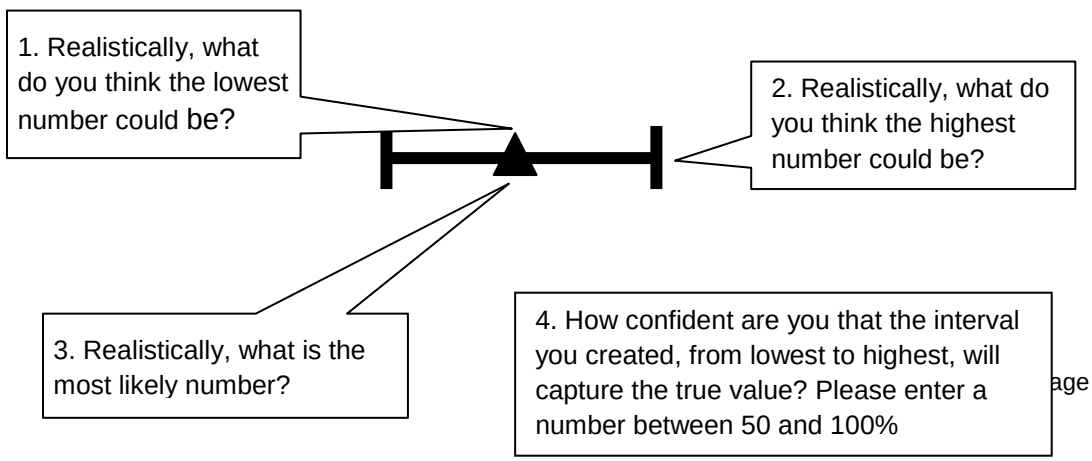

Figure 1. The 4-step interval elicitation process for collecting expert opinion (Speirs-Bridge et al., 2010).

For each expert elicitation exercise, a database with responses of the questionnaires was created in an Excel™ spreadsheet (PC/Windows XP, 2006). The 80% derived intervals were obtained using a LogNormal transformation of the experts intervals, as the experts' estimates were not considered normally distributed (Speirs-Bridge et al., 2010). To obtain a single estimate for each probability question to be included in the exposure assessment model, responses from all experts were considered and combined. As Vose (2008) indicates variability of the estimate should be incorporated in the model using a stochastic process and uncertainty of the estimates should be considered using uncertainty distributions for the model parameters. For the probability questions, each expert response (most likely, lowest and highest number of operations) was modelled using a Pert distribution. This distribution, which is frequently used to model expert opinion, is four times more sensitive to the most likely value than to the minimum and maximum values (Vose, 2008). To combine responses of all experts for each probability question and to incorporate the differences in expert opinions, a Discrete distribution was used. This distribution considers the probability estimate from each expert, which was obtained with a Pert distribution, and the weight given to each expert. An equal weight was used for all experts in the current assessments; however, experts' answers considered to be non-plausible and/or outliers were excluded from the analysis.

The specific methodology for eliciting expert opinion and the purpose of the exercise differed among parameters to be estimated as described below. Results from these elicitation exercises will be presented when describing the input parameters used for each model.

a. Sheep flock vaccination

Information about the proportion of flocks vaccinated for Ovine Johne's disease was elicited from District Veterinary Officers (DVO) attending the annual DVO meeting at Armidale

(NSW) on 19 - 21 March 2013. A questionnaire was developed (Appendix 2) to obtain information about (a) DVO's understanding of the minimum, most likely and maximum percentage of flocks vaccinated in each of the local government areas serviced by them; and (b) their confidence (from 50 to 100%) that the true percentage will fall within the minimum-maximum range. The questionnaire was distributed among DVOs participating in the conference and completed copies were collected at the conclusion of the conference afternoon session. A total of 16 district veterinarians participated in the exercise, with estimates for those with more than 5 years experience in the field and/or employed in the same role or similar being considered.

b. Disease pathology in sheep and cattle

Expert opinion about the ratio of multi- and pauci-bacillary sheep in a typical infected flock was obtained from Dr Douglas Begg, Senior Research Fellow at the Faculty of Veterinary Science, The University of Sydney. Dr Begg has extensive experience of Johne's disease pathology and has published about 25 peer-reviewed research articles on various aspects of the disease. A questionnaire was prepared (Appendix 3) to obtain information about (a) his understanding of the minimum, most likely and maximum proportion of multibacillary, paucibacillary and Perez 1 or 2 score sheep among 100 adult and 100 young infected sheep, separately in a non-vaccinated and a vaccinated flock; and (b) his confidence (from 50 to 100%) that the true proportion will fall within the minimum-maximum range. Dr. Begg's estimates described the proportions at the farm level; however, these estimates were modified considering that a proportion of multibacillary animals would not be sent to the abattoir, due to mortality or not being fit for transport. Dr. Begg was also asked to provide an estimate for this proportion of animals. In addition, Dr. Begg was consulted in relation to the probability of infected animals showing clinical signs.

The questionnaire was emailed to Dr Begg after clarifying the objective of the project. Dr Begg completed and returned the questionnaire by email.

Initially, the age groups of young and adult sheep were specified to be <2 year old and >2 year old in the questionnaire but later, after discussion with the project steering committee, the age classification for young sheep was changed to 12 months because that is the age group most commonly slaughtered for meat purposes. Dr Begg was requested to update his estimates based on the revised age specification for young sheep.

Literature was used to estimate the proportion of the different disease pathology categories in adult cattle; however, Dr. Begg was asked to provide estimates (minimum, most likely, maximum and confidence) for young animals as well as the proportion of multibacillary

animals that would not be sent to the abattoir (due to mortality and not being fit for transport as previously explained).

c. Antemortem inspection and pre-slaughter practices and activities at the abattoir

Expert opinion about the conduct of antemortem inspection and pre-slaughter practices and activities at a range of cattle and sheep abattoirs in eastern Australia was obtained from veterinarians with extensive abattoir experience. A questionnaire was developed (Appendix 4) to obtain information separately for cattle and sheep on:

- clinical signs of Johne's disease recognised by abattoir personnel
- % of times animals showing these clinical signs are separated from the lot in the lairage
- % of these separated animals that would be condemned and not used for human consumption
- measures used to reduce surface contamination prior to slaughter
- processes implemented for washing cattle pre-slaughter
- % of cattle lines that would undergo pre-slaughter wash
- criteria used to identify cattle for additional pre-slaughter wash treatment.

Although questions requesting percentages were structured to request minimum, most likely and maximum proportions, only the typical or most likely percentage was reported by most experts. Instead several experts provided qualitative statements about the frequency and reasons for separation of thin animals at antemortem. In this exercise the experts were not asked to state their level of confidence about reported percentages. This was considered appropriate as the standards for practices investigated are designated by regulatory standards such as the Australian Standard for the Hygienic Production and Transportation of Meat and Meat Products for Human Consumption (ANZFRM, 2007).

Nine phone interviews were conducted by one researcher with 2 Department of Agriculture Fisheries and Forestry (DAFF) Field Operations Managers, 4 On-Plant Veterinarians (OPV) at export abattoirs, 2 veterinarians recently retired from such roles and 1 university based veterinarian who regularly visits an export cattle abattoir for teaching and research purposes. The experts were required to have a minimum of 5 years experience working in Australian abattoirs. Each interview took 30-75 minutes depending on whether the expert had experience with cattle and/or sheep abattoirs. Across the interviews information was collected about the following types of abattoirs:

- Queensland – Cattle (3 abattoirs); Sheep (1 abattoir)

- New South Wales – Cattle (2); Sheep (1)
- Victoria – Cattle (2); Sheep (1)
- South Australia – Cattle (1); Sheep (1)
- Tasmania – Cattle (1).

d. Grams of faeces present in carcasses at the end of the slaughter line

A 4-step elicitation procedure was used to gather expert opinion on the grams of faeces present in sheep and cattle carcasses at the end of the slaughter line. A questionnaire was developed (Appendix 5) to investigate the minimum, most likely and maximum grams of faeces expected to be present in lambs, sheep, adult cattle (cows and bulls) and young cattle (steers and heifers). Information on Total Viable Counts (TVC) reported through the E. coli and Salmonella Monitoring Program (ESAM) was provided to the experts, who were asked to provide estimates for those abattoirs with the lowest and highest mean of TVC/ g of carcass. The ESAM program is a national surveillance program that operates at export abattoirs throughout Australia.

A total of seven members of the Scientific Risk Management Panel of the Food Safety program within Meat and Livestock Australia participated in this exercise which represented university, government and independent consultants. Their expertise covered the fields of microbiology, epidemiology and food safety, with all members having over 15 years of experience in these fields.

Experts were provided with the questionnaire and asked to bring their responses the following day. Responses were then collated and de-identified results shown to the experts, allowing them to have a discussion about the questions and results. At the end of the discussion time, experts were asked to reassess their estimates. These final estimates, which were not shown to the experts, were the ones used for this assessment. As previously explained, individual estimates were calculated using a Pert distribution using the most likely estimate and the 80% derived interval, and all experts were combined using a Discrete distribution. Results for the abattoirs with lowest and highest TVC were averaged.

3.3.3 Visits to abattoirs

Dr. Jonathan Webber has extensive expertise on the abattoir process given he is employed in a casual capacity by DAFF as an on-plant veterinarian in beef and pork abattoirs. Besides attending abattoirs for work purposes, during the project he visited two sheep abattoirs, in Tamworth and Wallangarra, to gain a better understanding of the slaughter process for sheep. Dr. Marta Hernandez-Jover attended the Tasmanian Quality Meat abattoir in Cressy,

Tasmania, a sheep abattoir actively involved with the control of Ovine Johne's disease among producers in the region.

3.4 Exposure assessment for the sheep industry

This section describes the models used for the different modules of the exposure assessment within the sheep industry, including the scenario trees developed as well as the input parameters used to populate these models.

3.4.1 On-farm

The on-farm module is a representation of the process by which a batch of animals is sent from one source farm direct to the abattoir and the composition of this batch in relation to MAP infection. The scenario tree used to investigate the on-farm module is shown in Figure 2. The output of this module is the probability of sheep within different MAP infection categories and within each geographical area and animal type (adult animals and young animals) being sent to the abattoir. These categories are:

- Non-infected animals from infected flocks
- Animals with low level of MAP infection (Paucibacillary animals) from infected flocks
- Animals with high level of MAP infection (Multibacillary animals) from infected flocks
- Non-infected animals from non-infected flocks

The nodes used in this scenario tree, as well as the input parameters and data sources used are described in Table 3. A detailed description of these nodes follows.

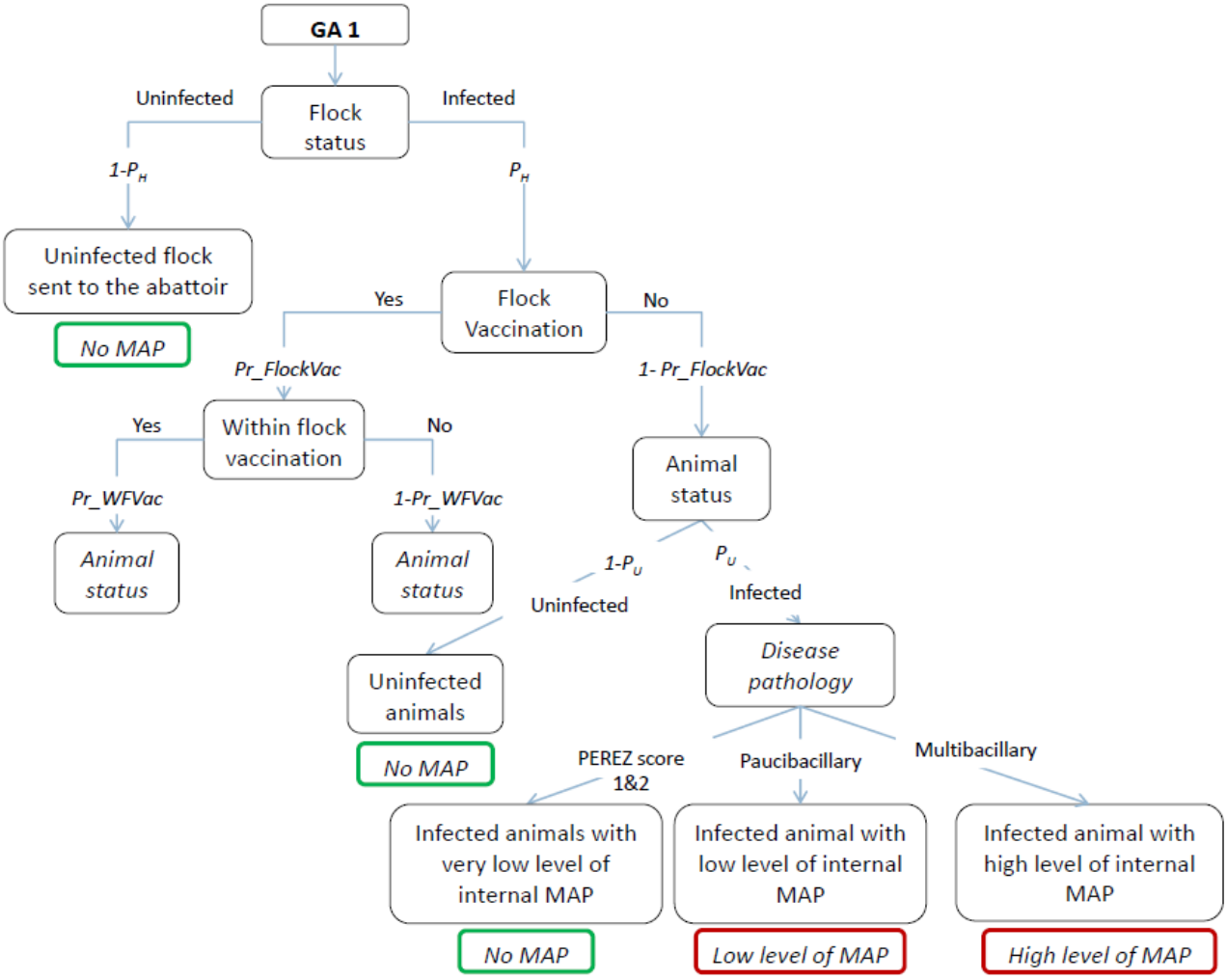

Figure 2. Sheep on-farm scenario tree *(GA1, geographical area 1, the same scenario was used for Geographical area 2 and 3; P_H, Flock prevalence; Pr_Flock Vacc, Proportion of flocks vaccinated; Pr_WFVac, Proportion of vaccinated animals within vaccinated flocks; P_U, Individual prevalence)*

Table 3. Nodes, parameter estimates and input values used for the on-farm component of the exposure of *Mycobacterium avium* subsps. *paratuberculosis* to human in sheep.

Node	Branch of the node	Parameter estimates	Input value	Data sources
1. Flock status	*Infected* *Uninfected*	Probability of a flock being infected (P_H) (different in each geographical area)	Beta distributions	Abattoir monitoring data for the 2012 calendar year, Central Animal Health Database (Ausvet, 2013)
2. Flock vaccination	*Yes* *No*	Proportion of vaccinated flocks within each geographical area (*Pr_FlockVacc*)	Individual expert opinion: Pert (lowest, most likely, highest) Combined expert opinion: Discrete (Outcome Pert, expert opinion weights) Output Discrete (median, 5-95%) GA1: 0 (0 – 0) GA2:0.704 (0.56 – 0.88) GA3: 0 (0 – 0.14)	Expert opinion – District veterinarians of Livestock Pest and Health Authority
3. Within flock vaccination	*Yes* *No*	Proportion of vaccinated animals within vaccinated flocks (*Pr_WFVacc*) (different depending on animal age)	Semiquantitative estimates Adult animals – High (Uniform (0.7,1)) Young animals – Very low (Uniform (0.001,0.05))	Expert opinion – Project Steering Group; Jeff Eppleston (Bathurst Livestock Pest and Health Authority)
4. Animal status	*Infected* *Uninfected*	Probability of an animal being infected within infected flocks (P_U) (different depending on vaccination status)	Based on MAP shedding: Non-vaccinated animals (median 0.0272; 95%CI, 0.014 – 0.0686) Vaccinated animals (0.0072; 0.0039 – 0.0127) Non-vaccinated animals in vaccinated flocks (average of non-vaccinated and vaccinated)	Dhand et al. (2013)
5. Disease pathology	*Perez Score 1&2* *Paucibacillary* *Multibacillary*	Proportion of infected animals within each disease pathology category (different depending on vaccination status and animal age)	Individual expert opinion: Pert (lowest, most likely, highest) Proportion of multibacillary animals that will not be sent to the abattoir (mortality, farmer awareness): Pert(0.3, 0.46, 0.60)	Perez et al. (1996); Sergeant, (2002) Expert opinion

3.4.1.1 Flock status

This node represents the probability of the flock, from which the batch of animals sent to the abattoir is sourced, being infected (P_H) in the three different geographical areas used in this assessment. The branches of this node are *Infected* and *Uninfected*. To estimate these probabilities, abattoir monitoring data for the 2012 calendar year, extracted from the Central Animal Health Database (National Johne's Disease Control Program, 2013) were used (Table 4). The median and 95% percentile prevalence estimates were obtained using a standardised Bayesian simulation method, which combines prior estimates of prevalence (from previous year) with estimates of flock-level sensitivity and specificity of abattoir monitoring and the observed results. Data are based on proportion of Property Identification Codes (PICs) infected.

Table 4: Summary of abattoir surveillance and corresponding prevalence estimates by State and prevalence area for 2012 (National Johne's Disease Control Program, 2013)

State	Prevalence Area	PIC results				Prevalence estimate	
		Negative	Positive	Total	% +ve	Median	95%-ile
New South Wales	High Prevalence	39	2	41	4.9	16.9	81.5
	Medium Prevalence	3	0	3	0	27.2	81
	Low Prevalence	399	0	399	0	0.6	1.7
Queensland	Low Prevalence	214	0	214	0	0.4	1.2
South Australia	Medium Prevalence	154	2	156	1.3	2.5	7.5
	Low Prevalence	1,656	9	1,665	0.5	0.5	1
Tasmania	Medium Prevalence	161	39	200	19.5	19.1	24
Victoria	High Prevalence	381	138	519	26.6	33.2	45.6
	Medium Prevalence	138	21	159	13.2	12.1	16.6
Western Australia	Medium Prevalence	259	17	276	6.2	5.4	7.8
Total PICs		3,404	228	3,632	6.3	0.5	2.3

The P_H estimates for the current assessment were incorporated into the model using Beta distributions based on the median and 95% percentiles. The median and 95 percentiles were used to extrapolate the two parameters required for the Beta distribution, alpha and beta, using an online epidemiology tool developed by AusVet Animal Health Services (http://epitools.ausvet.com.au/content.php?page=BetaParams1).

The following prevalence estimates were used to estimate the P_H in the different geographical areas (Table 5). An average between the outputs of the beta distributions for the different states within each geographical area was used.

Table 5. Prevalence estimates used to estimate Ovine Johne's Disease flock prevalence (P_H) in the different geographical areas considered in this assessment.

		Prevalence estimate		Beta parameters	
Geographical Area	State	Median	95%-ile	Alpha	Beta
GA1 (Extremely low prevalence)	Queensland	0.4	1.2	3.2	541.4
	New South Wales	0.6	1.7	3.4	402.5
GA2 (High prevalence)	Tasmania	19.1	24	41.2	171.4
	Victoria	33.2	45.6	15.8	30.8
	New South Wales	16.9	81.5	1.2	1.9
GA3 (Low prevalence)	Western Australia	5.4	7.8	20.0	334.2
	South Australia	2.5	7.5	3.0	80.5

3.4.1.2 Flock vaccination

This node represents the proportion of vaccinated flocks within each geographical area (*Pr_FlockVacc*). A flock can be vaccinated or unvaccinated. The expert opinion elicitation exercise previously described (*Section 3.3.2.2, a*) was used for estimating these proportions. The estimates for the proportion of vaccinated flocks used for each geographical area are described in Table 6.

Table 6. Estimates for the proportion of Ovine Johne's Disease vaccinated flocks in each geographical area considered in this assessment.

			Vaccination estimates	
Geographical Area	Experts (n)	Local government areas (n)	Median	5% – 95%-ile
GA1 (Extremely low prevalence)	1	1	0	0 – 0
GA2 (High prevalence)	4	14	0.704	0.56 – 0.88
GA3 (Low prevalence)	12	41	0	0 – 0.14

3.4.1.3 Within Flock vaccination

This node represents the proportion of vaccinated animals within vaccinated flocks and within a batch of animals being sent to the abattoir. The proportion of vaccinated animals within a batch of animals sent to the abattoir will be dependent on the type of animals in the batch. This assessment considers batches of adult sheep, as those animals older than 2 years of age, and young animals of approximately 12 months of age. To estimate the proportion of animals being vaccinated in each batch, consultation with the members of the

Project Steering Group (Appendix 1) and Dr Jeff Eppleston, district veterinarian from the Bathurst Livestock Pest and Health Authority was conducted. Qualitative estimates were obtained and subsequently transformed into quantitative estimates using uniform distribution following the semi-quantitative methodology described in the Guidelines for Import Risk Analysis (DAFF, 2004). For a vaccinated flock, the proportion of vaccinated adult sheep being sent to the abattoir was estimated to be *high* (Uniform (0.7,1.0) and for young animals this proportion was estimated to be *very low* (Uniform 0.001,0.05). It is believed that producers do not tend to vaccinate animals to be sent to the abattoir as young lambs, since clinical disease does not develop until the animal is approximately two years old.

3.4.1.4 Animal status

This node represents the probability of an animal being infected within infected flocks (P_U). Three probabilities were required for this node, according to the vaccination status: non-vaccinated animals in non-vaccinated flocks, vaccinated animals in vaccinated flocks and non-vaccinated animals in vaccinated flocks. These probabilities were estimated using a previously published study, evaluating the effectiveness of Gudair^{TM} vaccine in decreasing the prevalence of shedding of MAP in flocks of varying initial prevalence (Dhand et al., 2013). Thirty-seven self-replacing Merino flocks from New South Wales and Victoria that had been vaccinating lambs for at least five years were part of this study. Pre-vaccination prevalence in these flocks was estimated using results from pooled faecal culture, agar gel immunodiffusion or both tests. Post-vaccination prevalence was estimated from pooled faecal culture approximately five or more years after commencement of vaccination. A Bayesian model was developed to estimate and compare the pre- and post-vaccination prevalences for the enrolled flocks accounting for the sensitivity and specificities of the respective diagnostic test. Results from this study suggest that vaccination causes a significant decline in Ovine Johne's disease prevalence, from a pre-vaccination median prevalence of 2.72% (95% probability interval, 1.40 - 6.86%) to a post-vaccination median prevalence of 0.72% (0.39 - 1.27%). These prevalence estimates were used in the current assessment and incorporated into the model using Beta distributions with parameters extrapolated using the online epidemiology tool developed by AusVet Animal Health Services (http://epitools.ausvet.com.au/content.php?page=BetaParams1). Due to the lack of accurate data on the relationship between shedding and infection, shedding information has been used to estimate animal-level post-vaccination prevalence. As a consequence, the model might be underestimating the true animal-level prevalence, as it is known that a proportion of infected animals do not shed MAP.

3.4.1.5 Disease pathology

This node represents the proportion of infected animals within each disease pathology category (Perez Score 1 & 2, Paucibacillary and Multibacillary) in a batch of animals sent to the abattoir. This node is required, since the amount of MAP in faeces and product will differ according to the disease pathology category, and as such the potential probability of exposure of humans to MAP. Different proportions were required depending on the vaccination status and the type of animals sent to the abattoir (adult sheep, young animals), which were estimated using the expert opinion elicitation exercise previously described (*Section 3.3.2.2. b*) and literature (Perez et al., 1996; Sergeant, 2002).

Table **7** shows the parameter estimates used to calculate the proportion of each category within infected animals on the farm. To calculate these proportions for a batch of animals sent to the abattoir, the assessment considered that a proportion of multibacillary animals on-farm would not be sent to the abattoir, due to mortality or not being fit for transport. This proportion was estimated with expert opinion and incorporated into the model with a Pert distribution (Pert(0.30,0.46,0.60)).

The disease pathology categories used fit with a system for classification of lesions in cattle, sheep and goats applied by various research groups based on: presence and location of granulomatous lesions; cell types present; and presence and subjective assessment of MAP numbers in lesions (Perez et al., 1996; Corpa et al., 2000; Gonzalez et al., 2005). Perez Score 1 and 2 constitute small to multiple focal, well-demarcated granulomas in small intestine +/- mesenteric lymph nodes. In contrast, the paucibacillary category involves severe diffuse granulomatous enteritis of small intestine with MAP present sporadically in low numbers, and the multibacillary, also severe diffuse granulomatous enteritis of small intestine but with MAP present in large numbers in intestine and mesenteric lymph nodes.

Table 7. Parameter estimates used to calculate the proportion of the different disease pathology category within infected animals on the farm according to the vaccination status and animal age.

	Adult sheep (> 2y old)			Young animals (12 mo old)		
	Perez 1 or 2	Paucibacillary	Multibacillary	Perez 1 or 2	Paucibacillary	Multibacillary
Non-vaccinated animals						
Most likely	0.60	0.15	0.25	0.925	0.05	0.025
Minimum	0.33	0.03	0.03	0.379	0.03	0.03
Maximum	0.93	0.47	0.38	0.94	0.44	0.33
Vaccinated animals						
Most likely	0.90	0.05	0.05	0.97	0.02	0.01
Minimum	0.61	0	0	0.91	0	0
Maximum	1.0	0.17	0.23	1.0	0.06	0.06

3.4.2 Lairage

The lairage module is a representation of the process starting with a batch of animals arriving at the abattoir lairage directly from the farm and finishing when these animals enter the slaughter line. While in the lairage, the composition of the batch of animals in relation to MAP infection might change due to cross-contamination and separation of clinically infected animals. The scenario tree used to investigate the lairage module is shown in Figure 3.

The output of this module is the probability of sheep within different MAP infection categories and within each geographical area and animal type (adult animals and young animals) being processed in the slaughter line. These categories are:

- Non-infected animals from non-infected flocks (the same as for the on-farm output)
- Infected flocks:
    - Main lot:
        - Animals with no MAP infection (non-infected and Perez 1 or 2) and low levels of external MAP
        - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
        - Animals with high level of MAP infection (Multibacillary) and high level of external MAP

- Separated lot (during antemortem inspection activities):
  - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
  - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
  - Condemned animals

The nodes used in this scenario tree, as well as the input parameters and data sources used are described in Table 8. The first node (MAP presence) is the output from the on-farm model. A detailed description of the rest of the nodes follows.

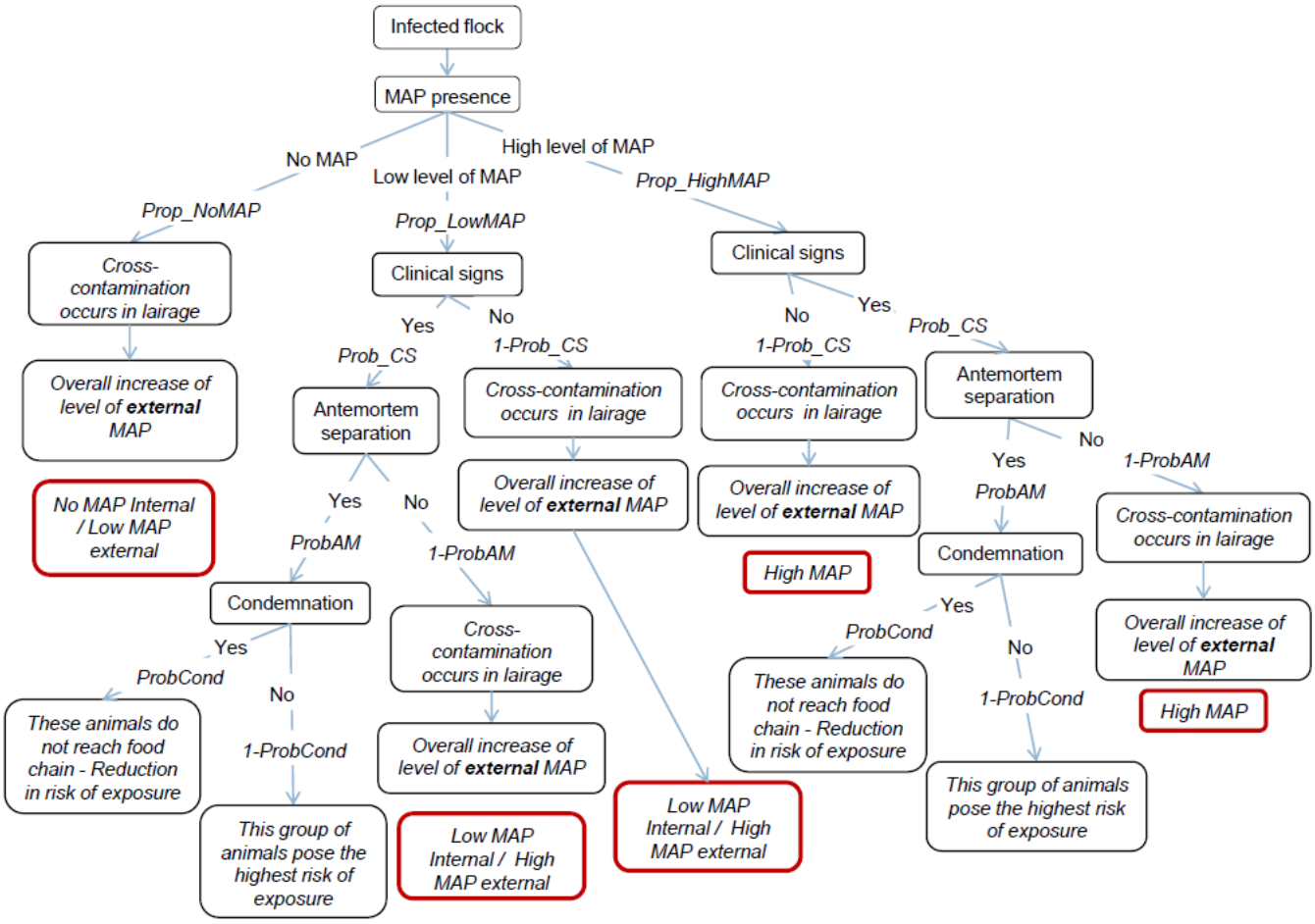

Figure 3. Sheep lairage scenario tree *(Prop_NoMAP, Proportion of non-infected animals; Prop_LowMAP, Proportion of animals with low level of MAP; Prop_HighMAP, Proportion of animals with high level of MAP; Prob_CS, Proportion of animals showing clinical signs; ProbAM,* Probability of detection and separation of clinically affected animals; *ProbCond*, Probability of condemnation of clinically affected animals separated at antemortem*)*

Table 8. Nodes, parameter estimates and input values used for the lairage component of the exposure of *Mycobacterium avium* subsps. *paratuberculosis* to human in sheep.

Node	Branch of the node	Parameter estimates	Input value	Data sources
1. MAP presence	*No MAP – non-infected flocks* *No MAP* *Low level of MAP* *High level of MAP*	Proportion of sheep within a batch arriving at the abattoir with the different level of MAP infection	-	Output from the on-farm scenario
2. Clinical signs	*Yes* *No*	Probability that multibacillary and paucibacillary animals showing clinical signs (*Prob_CS*)	Paucibacillary animals (1%): Beta(2,100) Multibacillary animals (10%): Beta(11,91)	Expert opinion
3. Antemortem detection and separation	*Yes* *No*	Probability of clinically affected animals being detected and separated during antemortem inspection at the abattoir (*ProbAM*)	Semiquantitative estimate Very low (Uniform (0.001, 0.05)	Expert opinion: On-plant veterinarians of export abattoirs
4. Condemnation	*Yes* *No*	Probability of clinically affected animals being condemned after detection and separation (*ProbCond*)	Semiquantitative estimate Low (Uniform (0.05,0.3)	Expert opinion: On-plant veterinarians of export abattoirs

3.4.2.1 Clinical signs

This node accounts for the probability of paucibacillary and multibacillary animals being sent to the abattoir showing clinical signs (*Prob_CS*) and is incorporated into the lairage model as only those animals showing clinical signs could be detected and separated during antemortem inspection activities. Ovine JOHNE'S DISEASE causes non specific clinical signs, with a progressive weight loss and emaciation in older animals being the most common signs. Diarrhoea is usually absent or limited to soft, pasty faeces (Cousins et al., 2002). Due to the progressive nature of the disease, only a proportion of animals with multibacillary and paucibacillary infection show clinical signs, and this proportion would be higher among multibacillary than paucibacillary animals. In addition, these proportions might be lower among animals being sent to the abattoir as some of the affected animals with severe clinical signs might not be sent to the abattoir. The probability of animals being sent to the abattoir showing clinical signs was estimated to be approximately 10% and 1% for multibacillary and paucibacillary animals, respectively, after consultation with Dr. Begg. A beta distribution was used to incorporate these proportions into the model.

3.4.2.2 Antemortem detection and separation

3.4.2.3 Condemnation

These two nodes represent the probability of clinically affected animals being detected and separated during antemortem inspection activities at the lairage (*ProbAM*) and the probability of clinical affected animals being condemned after detection and separation (*ProbCond*). Parameters used for these nodes were estimated using expert opinion (*Section 3.3.2.2, c*). According to the qualitative responses of four experts on the probability of Ovine Johne's disease infected animals being separated during antemortem inspection and condemned, *ProbAM* and *ProbCond* were estimated to be *very low* and *low*. These estimates were based on the main aspects discussed by the four experts who provided input on the sheep lairage questions, which could be summarized as follows:

- Stockmen are not aware of the clinical signs of Ovine Johne's disease, and they might only be aware of the disease if in a high prevalence area
- Sheep are inspected in a mob and not individually
- Visual assessment of emaciation is not effective in woolly sheep and antemortem inspection does not require touching the animals
- Only severely thin animals deemed as No Commercial Value would actually be separated based on clinical signs at antemortem, and these are excluded from the food chain and usually sent to rendering
- There is a large number of adult sheep sent to the abattoir that are very thin
- In lambs, separation of very thin animals might be a bit more likely as is not that common to see very thin lambs
- Condemnations under disposition of emaciation usually occur during postmortem inspection rather than antemortem inspection
- Condemnation based on antemortem inspection might occur in animals with No Commercial Value, severe emaciation and welfare concerns.

The qualitative estimates were transformed into quantitative estimates using uniform distributions following the semi-quantitative methodology described at the Guidelines for Import Risk Analysis (DAFF, 2004).

3.4.3 Abattoir

The abattoir module is a representation of the process starting with a batch of animals entering the slaughter line and finishing with the different types of products originating from these animals. This module is based on a simulation model, with different batches of 50 animals coming from the lairage (three geographical areas, two types of animals, antemortem separation) going through the slaughter line. This model accounts for the potential cross-contamination among carcasses due to the amount of faeces present on the carcass surface. As such, each carcass has estimated quantities of internal MAP presence as well as external MAP contamination. The amount of internal MAP will depend on the animal level of infection. The external MAP contamination will be due to faeces coming from the intestines of the animal itself, which depends on the animal level of infection; and from faecal contamination of the hide, which depends on the level of infection of the animal and of other animals in the batch.

Figure 4 is a representation of the origin of MAP presence in each carcass going through the slaughter line. The parameters used for this simulation model are described in Table 9. The first parameter (MAP presence by consignment type) is the output from the lairage model. A detailed description of the rest of the nodes follows.

The output of this module is the average amount of MAP (in Log10) in a serving size of the product for each animal within a consignment. For each geographical area and type of animal (adult vs. young sheep), different products were considered. As previously described, these products for sheep were: skeletal muscle (trim), whole muscle (leg of lamb), liver and intestines.

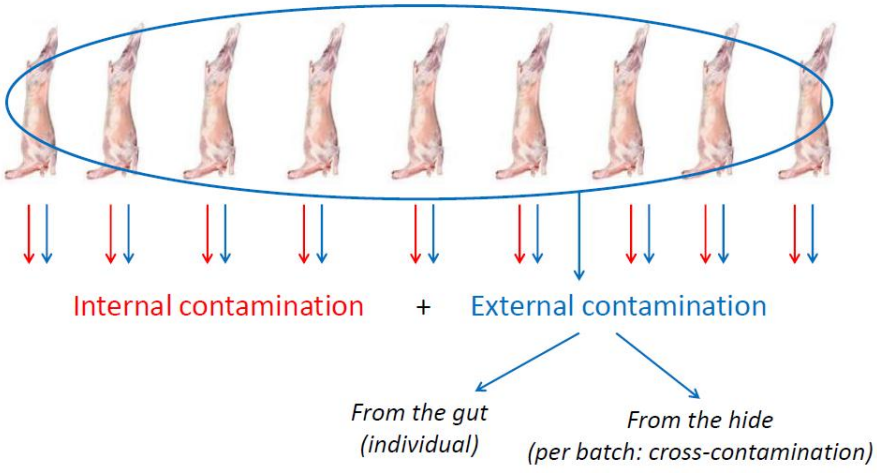

Figure 4. Representation of the origin of the *Mycobacterium avium* subspecies *paratuberculosis* present in each carcass going through the slaughter line.

3.4.3.1 Transfer of external MAP to carcass surface

This parameter accounts for: the probability of faecal matter being transferred onto the carcass surface; the grams of faeces being transferred; the proportion of faeces originating from hide and intestines; and, the amount of MAP present in faeces. The expert opinion elicited during the consultation process with the Scientific Risk Management Panel of the Food Safety program within Meat and Livestock Australia (*Section 3.3.2.2., d*) was used as the source to estimate the required inputs for this parameter.

Probability of transfer of faecal matter onto the carcass surface: This probability was assumed to be 1, after consultation with the expert panel, as transfer of some faecal matter from the intestines and hide to the carcass surface will occur independently of the hygienic practices applied at the abattoir.

Grams of faeces transferred: According to the output of the expert elicitation exercise, the grams of faeces present on the carcass surface of adult and young sheep was defined by the following distributions (median, 5-95%):

- Sheep carcasses: 0.403 (0.010 – 1.722)
- Lamb carcasses: 0.415 (0.026 – 1.672)

Proportion of faeces from hide and intestines: The proportion of faeces present on the carcass surface originating from hide was estimated by experts as (median, 5-95%) 0.86 (0.57 – 0.96). The proportion of faeces originating from intestines was extrapolated from the proportion originating from hide.

Amount of MAP in faeces: This parameter estimates the number of MAP (Colony-forming unit, CFU) per gram of faeces of infected animals. Only those animals with paucibacillary and multibacillary disease pathology were considered to be shedding MAP in faeces.

Previously published literature and results from unpublished studies have been used to estimate the CFU of MAP per gram of faeces in paucibacillary and multibacillary animals. However, there are limited studies with specific information on MAP shedding according to the animal disease pathology. A study using seven sheep, aged 2 or more years, with presence of acid-fast bacilli in their faeces, reported an average number of viable bacteria of 1.09×10^8 per gram of faeces excreted (Whittington et al., 2000). In another study by Whittington et al. (2001), the mixture of faeces of two sheep with multibacillary lesions, contained 1.2×10^6 viable MAP per gram. Reddacliff et al. (2006) investigated the efficacy of a killed vaccine for the control of Ovine Johne's disease in Australia, and reported shedding of 1.3 and 3.4×10^9 MAP per gram of faeces, for unvaccinated sheep older and younger than 2 years of age, respectively. Shedding was reduced among vaccinated sheep to 2.6×10^7 and 2.24×10^8 MAP per gram of faeces for sheep older and younger than 2 years of age, respectively.

Other studies report more specific information on MAP shedding in paucibacillary and multibacillary animals. According to these experimental studies, multibacillary animals have significantly higher levels of MAP in faeces (10 times higher or more) than paucibacillary animals as early as 4 months post inoculation. Results from these studies suggest that at 12 months post inoculation the estimated CFU MAP is 10^3 and 10^8 per gram of faeces for paucibacillary and multibacillary animals, respectively (Kawaji et al., 2011)(Begg and Plain, personal communication). These estimates were used in the current assessment.

3.4.3.2 Probability of disseminated infection

This parameter was incorporated into the model as not all infected animals will have disseminated MAP infection in tissues other than the gastrointestinal tract, and as such, it affected the probability of MAP being present in the final product considered in this assessment as well as the amount of MAP in product. Results reported in previously published studies were used in this assessment. Bower et al. (2011) reported approximately 7% of paucibacillary sheep with disseminated infection in liver, hepatic lymph nodes and blood. This percentage increased to approximately 70% for multibacillary animals. Reddacliff et al. (2010) reported 59% of sheep with clinical paratuberculosis with disseminated infection in muscle and 85% in peripheral lymph nodes. For subclinical sheep, only 4.5% and 32% of animals had disseminated infection in muscle and peripheral lymph nodes. Similar percentages were reported by Smith et al. (2011), with 71% of Johne's disease clinically affected sheep having MAP in muscle, while only 13% of non-clinically affected sheep.

The current assessment estimated the probability of disseminated infection in the different products considered in this assessment from these previous studies, using Beta distributions

to account for uncertainty and variability around these estimates, as described in

Table 9. It was assumed that all paucibacillary and multibacillary animals would have MAP in the intestines.

3.4.3.3 Amount of MAP in product

Among those animals with disseminated infection, the amount of MAP in a serving size of each product considered in this assessment (100g for muscle and liver; 10g for intestines) was estimated from a study by Reddacliff et al (2010). This study reported a mean of 46.8 CFU MAP (8.3 s.d.)/ g of muscle, among 37 Johne's disease clinically affected sheep. The only sheep with subclinical paratuberculosis and disseminated infection in muscle had 7.6 CFU MAP/g of tissue. Regarding lymph nodes, this study reported a mean of 114.8 CFU MAP (4.9 s.d.) / g of tissue, among 76 Johne's disease clinically affected sheep. Among 14 non-clinical sheep with disseminated infection in the lymph nodes, the mean CFU MAP / g of tissue was 42.6 (2.7 s.d.). These values were incorporated into the model using probability distributions, as indicated in Table 9, to incorporate uncertainty around the estimates.

For skeletal (trim) and whole muscle (leg of lamb) and liver, this model accounts for the amount of MAP in tissue as well as the lymph nodes present in the tissue. As such, estimates on the amount of lymph nodes in each of these products were required. Since no information was available on this parameter, this model used the following assumed parameters:

- Skeletal muscle (1 kg): Minimum, 0.5; Most likely, 1; Maximum, 2.
- Whole muscle (2 kg): Minimum, 2; Most likely, 5; Maximum, 7.
- Liver (200 – 400 grams): Minimum, 0.5; Most likely, 1; Maximum, 2.

These values were incorporated into the model using Pert distributions to account for uncertainty around these estimates, and the amount for a serving size of 100 grams was used.

The amount of MAP in liver tissue was assumed to be the same as that in muscle, and the amount of MAP in intestines was assumed to be the same as that in faeces.

Table 9. Input parameter and input values used for the slaughter floor simulation component of the exposure of *Mycobacterium avium* subsps. *paratuberculosis* (MAP) to human in sheep

Node	Parameter estimates	Input value	Data sources
1. MAP presence by consignment type	Proportion of sheep within a consignment in each level of MAP infection	-	Output from the lairage scenario
2. Transfer of external MAP to carcass surface	Probability of transfer of faecal matter onto the carcass surface Grams of faeces transferred Proportion of faeces from hide and intestines	Point estimate = 1 Individual expert opinion: Pert (lowest, most likely, highest) Combined expert opinion: Discrete (Outcome Pert, expert opinion weights) Output Discrete (median, 5-95%)	ESAM data Expert opinion
	CFU MAP Log10 / g of faeces	Paucibacillary: 3 CFU MAP Log10/g Multibacillary: 8 CFU MAP Log10/g	Reddacliff et al. (2006); Whittington et al. (2000, 2001); D. Begg (personal communication)
3. Probability of disseminated infection	Probability of infected animals having a disseminated infection in muscle, lymph nodes, liver and intestines (different according to disease pathology)	Probabilities incorporated with Beta distributions Paucibacillary: Muscle, 0.07; Lymph node, 0.30.; Liver, 0.07; Intestines, 1. Multibacillary Muscle, 0.70; Lymph node, 0.85.; Liver, 0.70; Intestines, 1.	Bower et al. (2011)
4. Amount of MAP in product	CFU MAP Log10/ g of product (lymph node, muscle, liver, intestines)	Paucibacillary: Muscle, Pert(0.62,0.88,1.14); Lymph node, Normal(1.63,0.43); Liver, Normal (1.63,0.43); Intestines (as faeces) Multibacillary: Muscle, Normal(1.67,0.92); Lymph node, Normal(2.06,0.69); Liver, Normal (2.06,0.69); Intestines (as faeces)	Reddacliff et al. (2010)

3.4.4 Sensitivity analyses

The sensitivity of some model outputs to variation of some of the input parameters was investigated using the @Risk Advanced Sensitivity Analysis (@RISK 6.0, Palisade Corporation, USA), to identify which input parameters were more influential on the outputs. The influence of each input parameter on the model outputs was evaluated by simulating the outputs using a series of fixed values for a given input variable. This process supports the identification of potential mitigation strategies. Within the geographical area with higher flock prevalence (geographical area 2, GA2), the following input parameters were investigated: Flock vaccination (*Pr_FlockVacc*), within flock vaccination (*Pr_WFVacc*), probability of sending multibacillary animals to the abattoir (related to the disease pathology node of the on-farm scenario) and the probability of antemortem detection (*ProbAM*). The outputs monitored were the probability of animals with high MAP infection being sent to the abattoir, and entering the slaughter line. Moreover, the influence of these parameters on the amount of MAP in final product was also investigated in addition to the amount of MAP per grams of faeces. In geographical areas with very low flock prevalence, the effect of increasing the flock prevalence on the outputs of the models was also evaluated.

Proportion and probability input parameters were allowed to vary from 0 to 1 in tenths (0.1, 0.2, 0.3...). Each of the values for each input parameter was evaluated separately in a simulation of 5,000 iterations, whilst values for all other input variables were fixed to the base value.

3.5 Exposure assessment for the cattle industry

This section describe the models used for the different modules of the exposure assessment within the cattle industry, including the scenario trees developed as well as the input parameters used to populate these models.

3.5.1 On-farm

Similar to sheep, the on-farm module is a representation of the process by which a batch of animals is sent from one source farm direct to the abattoir and the composition of this batch in relation to MAP infection. The scenario tree used to investigate the on-farm module is shown in

Figure 5 The output of this module is the probability of cattle within different MAP infection categories and within each scenario (prevalence area and animal type) being sent to the abattoir. These categories are:

- Non-infected animals from infected herds
- Animals with low level of MAP infection (Paucibacillary animals) from infected herds
- Animals with high level of MAP infection (Multibacillary animals) from infected herds
- Non-infected animals from non-infected herds

The nodes used in this scenario tree, as well as the input parameters and data sources used are described in Table 10, and a detailed description is provided below.

3.5.1.1 Herd status

This node represents the probability of the herd, from which the batch of animals sent to the abattoir is sourced, being infected (P_H) in the five different scenarios considered in this assessment (*Section 3.2.1; Table 2*). The branches of this node are *Infected* and *Uninfected*. The probability of a herd being infected in each of the scenarios was estimated using information on known infected beef cattle and dairy herds and apparent prevalence in Australia at August 2009, provided by Animal Health Australia (AHA, personal communication). It should be noted that this data is likely to underestimate the true herd-prevalence, particularly in dairy herds, due to the lack of tracing within the dairy industry and the fact that data relies on reported cases. After discussion with the Project Steering Group, the total number of herds used as denominators to calculate the apparent prevalence was adjusted using information provided by Dr. Evan Sergeant. Dr. Sergeant provided data on total number of unique PICs per state in Australia, based on an analysis of approximately 27 million NLIS records for individual cattle killed at abattoirs from 2005 to 2009. The proportion of infected herds was incorporated into the model using Beta distributions as detailed in Table 10.

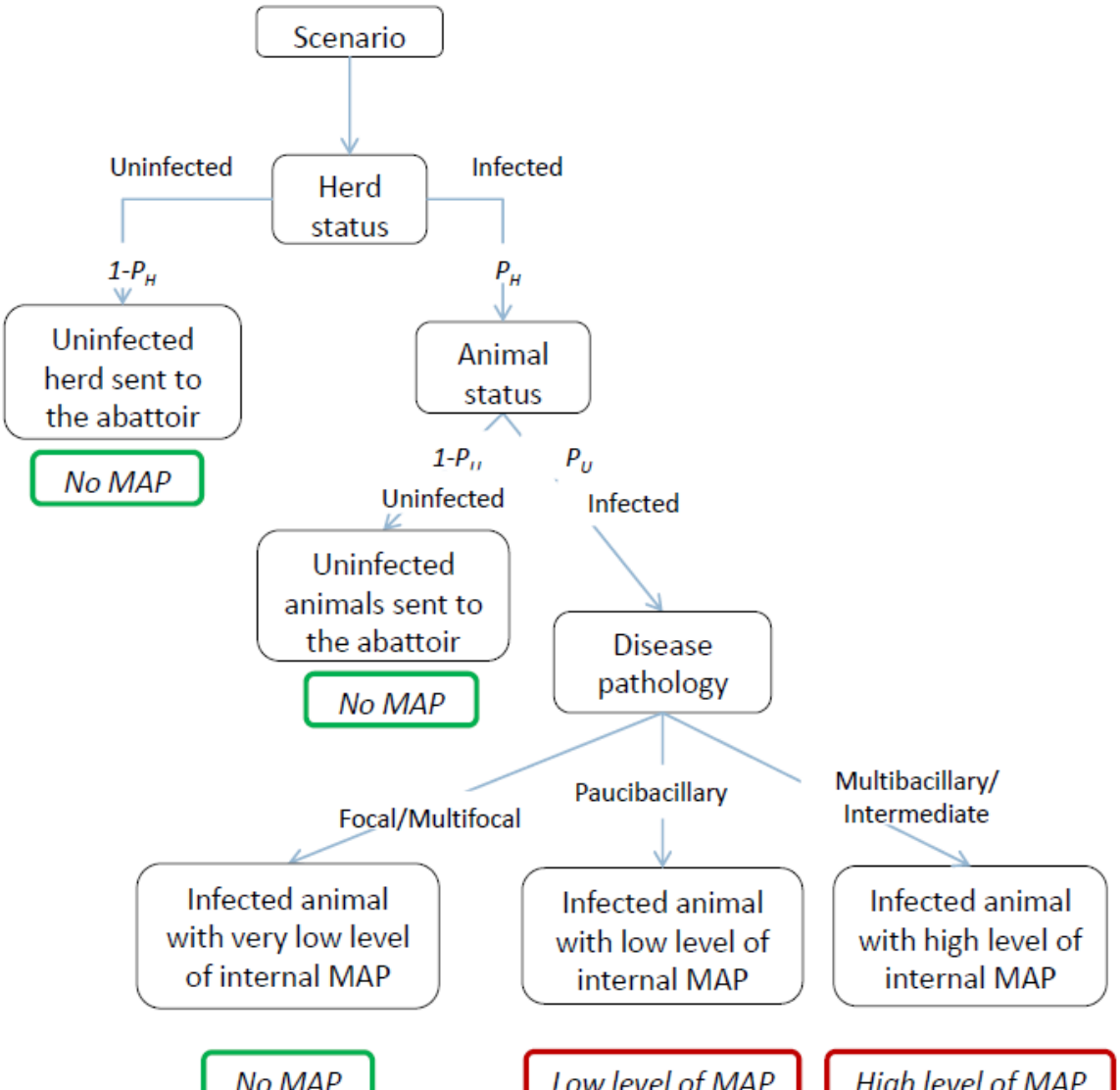

Figure 5. On-farm cattle scenario tree (P_H, *Herd prevalence;* P_U, *Individual prevalence)*

Table 10. Nodes, parameter estimates and input values used for the on-farm component of the exposure of *Mycobacterium avium* subsps. *paratuberculosis* to human in cattle.

Node	Branch of the node	Parameter estimates	Input value*	Data sources
1. Herd status	*Infected* *Uninfected*	Probability of a herd being infected (P_H) (different in each scenario)	Beta distributions : S1: 0/27,000; S2: 64/63,201; S3: 136/1176; S4: 1,018/5,044; S5: 0/670	Animal Health Australia (2012)
2. Animal status	*Infected* *Uninfected*	Probability of an animal being infected within infected herds (P_U)	Beef: Uniform(0.0077,0.0328) Dairy:Uniform(0.0174,0.0471)	Eltholth et al. (2009); Jubb (2004); Vangeel et al. (2012); Cattle MAP
3. Disease pathology	*Focal / Multifocal* *Paucibacillary* *Multibacillary*	Proportion of infected animals within each disease pathology category (different depending on animal age)	Beta distributions: *Adult cattle:* No lesions, focal, multifocal: 132/167 Paucibacillary: 3/167 Multibacillary: 32/167 *Young cattle:* No lesions, focal, multifocal: Pert(0.9,0.98,1) Ratio Paucibacillary:Multibacillary= 1:10 Proportion of multibacillary animals will not be sent to the abattoir (mortality, farmer awareness): Pert(0.3, 0.46, 0.60)	Gonzalez et al. (2005) Expert opinion

*S1 = Scenario 1, Beef, Beef Protected and Free zone; S2 = Scenario 2, Beef, Beef Protected and Management areas; S3: Dairy, Beef Protected zone; S4 = Scenario 4, Dairy, Management areas; S5 = Scenario 5, Dairy, Protected zone)

3.5.1.2 Animal status

This node represents the probability of an animal being infected within infected herds (P_U), which was considered to be different for beef cattle and for dairy herds. Very limited information on the within-herd prevalence of Johne's disease in dairy and beef cattle herds in Australia is currently available. As such, different sources were used to estimate the probabilities required for this node. Two studies investigated the effect a test and control program for Johne's disease in Victorian beef and dairy herds from 1992 to 2002. Among beef herds, an average individual prevalence of 0.77% at the first whole-herd test round was reported (Jubb and Galvin, 2004b). Among dairy herds, the within-herd prevalence was higher, with an average of 1.7% being reported (Jubb and Galvin, 2004a). In a systematic review on contamination of food products with MAP by Eltholth et al. (2009), 21 studies investigating individual and herd prevalence were reviewed. The mean individual prevalence reported in these studies was 3.3% and 4.7% in beef and dairy herds, respectively. A recent study investigating the seroprevalence of Johne's disease in cattle in Belgium in 2009-2010, reported a within-herd prevalence of 1.2% and 3.9% for beef and dairy herds, respectively.

Data gathered within the Australian Johne's disease Market Assurance Program for Cattle (CattleMAP) program in 2008 were reviewed and compared with estimates from the literature. Within dairy herds, the mean within-herd prevalence reported was 3.0%.

Due to the paucity of current data in Australia, the current assessment has used uniform distributions to account for the uncertainty and variability of results obtained in previous studies. Thus, the within-herd prevalence for beef and dairy herds was incorporated into the model as Uniform(0.0077,0.0328) and Uniform(0.0174,0.0471), respectively.

3.5.1.3 Disease pathology

This node represents the proportion of infected animals within each disease pathology category in a batch of animals sent to the abattoir. This node is required, since the amount of MAP in faeces and product will differ according to the disease pathology category, and as such the potential probability of exposure of humans to MAP. Different proportions were required depending on the type of animals sent to the abattoir (adult cattle from dairy herds, young cattle from beef herds). Previous published literature (Gonzalez et al., 2005) and expert opinion (*Section 3.3.2.2. b*) were used to describe the disease pathology categories used in this assessment and to estimate these input parameters; however, the different disease pathology categories in cattle are not as well defined as in sheep. Gonzalez et al. (2005) conducted a study investigating the histopathological classification of lesions associated with natural Johne's disease infection among 167 infected adult cattle. Among these, 51 cows had no lesions with the rest having different types of lesions, including: 68 focal (58.6%), 13 multifocal (11.2%), 15 diffuse multibacillary (12.9%), 3 diffuse lymphocytic (2.6%) and 17 diffuse intermediate (14.7%). A description of these lesions according to these authors is provided below.

- Focal: Small, well-demarcated granulomas formed by macrophages in the lymphoid tissue in small intestine and mesenteric lymph nodes.
- Multifocal: Multiple focal, well-demarcated granulomas in the lymphoid tissue and lamina propria of small intestine.
- Diffuse multibacillary: Severe diffuse granulomatous enteritis of small intestine characterised by macrophages, and MAP present in large numbers in intestine and mesenteric lymph nodes.
- Diffuse paucibacillary: Severe diffuse granulomatous enteritis of small intestine characterised by lymphocytes, and MAP present sporadically and in low numbers.
- Diffuse intermediate: Diffuse granulomatous enteritis of small intestine with infiltrate containing abundant lymphocytes, plasma cells, giant cells and macrophages. MAP

are present in relation to number of macrophages but in lesser numbers than multibacillary form.

This assessment assumes that animals with focal and multifocal lesions pose a negligible risk of exposure of humans to MAP and as such these categories are considered together with the proportion of animals with no lesions. In addition, diffuse intermediate and diffuse multibacillary were assumed to pose a similar risk and considered within the same category. As such, the proportions of animals within each disease pathology category used in the current assessment are: 132/167 no lesions, focal and multifocal; 3/167 paucibacillary; 32/167 multibacillary. These proportions were incorporated into the model using Beta distributions.

For young cattle, according to the expert opinion, the most likely proportion of animals with no lesions, focal or multifocal lesions was estimated to be 0.98, with a minimum of 0.90 and a maximum of 1. This proportion was incorporated into the model using a Pert distribution. To estimate the proportion of paucibacillary and multibacillary animals, the ratio between both categories reported by Gonzalez et al. (2005) was used.

As for the sheep assessment, to calculate these proportions for a batch of animals sent to the abattoir, the assessment considered that a proportion of multibacillary animals on-farm would not be sent to the abattoir, due to mortality or not being fit for transport. The same proportion as for the sheep assessment was used and incorporated into the model with a Pert distribution (Pert(0.30,0.46,0.60)).

3.5.2 Lairage

The lairage module is a representation of the process starting with a batch of animals arriving to the abattoir lairage directly from the farm and finishing when these animals enter the slaughter line. As for sheep, while in the lairage, the composition of the batch of animals in relation to MAP infection might change due to cross-contamination and separation of clinically infected animals. The scenario tree used to investigate the lairage module in cattle is the same than the one used for sheep (Figure 3).

The output of this module is the probability of cattle within different MAP infection categories and within each scenario (adult animals from dairy herds and young cattle from beef herds) being processed in the slaughter line. These categories are:

- Non-infected animals from non-infected herds (the same as the on-farm output)
- Infected herds:
    - Main lot:

- Animals with no MAP infection (non-infected and no lesions, focal, multifocal) and low levels of external MAP
- Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
- Animals with high level of MAP infection (Multibacillary) and high level of external MAP

- Separated lot (during antemortem inspection activities):
  - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
  - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
  - Condemned animals

The nodes used in this scenario tree, as well as the input parameters and data sources used are described in Table 11. The first node (MAP presence) is the output from the on-farm model. A detailed description of the rest of the nodes follows.

Table 11. Nodes, parameter estimates and input values used for the lairage component of the exposure of *Mycobacterium avium* subsps. *paratuberculosis* to human in cattle.

Node	Branch of the node	Parameter estimates	Input value	Data sources
1. MAP presence	*No MAP – non-infected herds* *No MAP* *Low level of MAP* *High level of MAP*	Proportion of cattle within a batch arriving at the abattoir with the different level of MAP infection	-	Output from the on-farm scenario
2. Clinical signs	*Yes* *No*	Probability that multibacillary and paucibacillary animals showing clinical signs (*Prob_CS*)	Paucibacillary animals: Uniform(0,0.12) Multibacillary animals: Uniform(0.33,0.70)	Dennis et al. (2008)
3. Antemortem detection and separation	*Yes* *No*	Probability of clinically affected animals being detected and separated during antemortem inspection at the abattoir (*ProbAM*)	Semiquantitative estimate Very low (Uniform (0.001, 0.05)	Expert opinion: On-plant veterinarians of export abattoirs
4. Condemnation	*Yes* *No*	Probability of clinically affected animals being condemned after detection and separation (*ProbCond*)	Semiquantitative estimate Low (Uniform (0.05,0.3)	Expert opinion On-plant veterinarians of export abattoirs

3.5.2.1 Clinical signs

This node accounts for the probability of paucibacillary and multibacillary animals being sent to the abattoir showing clinical signs (*Prob_CS*) and is incorporated into the lairage model as only those animals showing clinical signs could be detected and separated during antemortem inspection activities. Johne's disease in cattle causes a chronic and progressive syndrome with emaciation and persistent diarrhoea; however, affected animals usually appear to be bright and alert until advanced stages of the disease (Cousins et al., 2002). Limited information on the occurrence of clinical signs of disease for the different disease pathology categories is available and as such, there is significant uncertainty about the parameters used for this node. Dennis et al. (2008), investigated the severity of enteric granulomatous inflammation in 40 MAP infected adult dairy cows, and reported higher proportion of animals with clinical signs among those animals with a more severe intestinal inflammation. Four intestinal inflammation grades were used based on histopathology observations, and clinical signs were recorded for each of these grades as described in

Table 12. These proportions were incorporated into the model as Uniform(0,0.12) and Uniform(0.33,0.70) for paucibacillary and multibacillary animals, respectively.

Table 12. Association of intestinal inflammation grades and clinical signs in adult dairy cattle (Dennis et al., 2008)

Intestinal inflammation grade	**Definition**	**Animals**	**Animals with clinical signs**
0	No inflammation/lesion	17	2 (12.0%)
1 – low severity	Scattered individual Langhans-type multinucleate giant cells and/or tiny clusters or epithelioid macrophages throughout the lamina propria or Peyer's patches of intestinal sections, or in the paracortex of lymph node sections	5	0 (0)
2 – moderate severity	Groups of epithelioid macrophages of varied size, uncommonly admixed with multinucleate giant cells / leukocytes dispersed in the submucosa and lamina propria	6	2 (33.3%)
3 - severe	Numerous coalescing groups of confluent sheets of epithelioid macrophages; multinucleate giant cells were rarely identified	10	7 (70.0%)

3.5.2.2 Antemortem detection and separation

3.5.2.3 Condemnation

These two nodes represent the probability of clinically affected animals being detected and separated during antemortem inspection activities at the lairage (*ProbAM*) and the probability of clinical affected animals being condemned after detection and separation (*ProbCond*). Parameters used for these nodes were estimated using expert opinion (*Section 3.3.2.2, c*) and results are very similar to those presented for the sheep assessment. According to the qualitative responses of eight experts on the probability of Johne's disease infected animals being separated during antemortem inspection and condemned, *ProbAM* and *ProbCond* were estimated to be *very low* and *low*. These estimates were based on the main aspects discussed by the eight experts who provided input on the cattle lairage questions, which could be summarized as follows:

- Stockmen are not aware of the clinical signs of Bovine Johne's disease, and they might only be aware of the disease if in a high prevalence area
- Lack of specificity of clinical signs - even the On-plant veterinarian is unlikely to separate Bovine Johne's disease infected animals
- Poor body condition on its own is not a sign for animals to be separated into suspect yard unless severe or accompanied other signs of illness or of welfare concerns.
- Only those animals deemed as No Commercial Value would actually be separated based on clinical signs at antemortem, and these are removed from the food chain and usually sent to rendering
- There is a large number of cows sent to the abattoir that are very thin (in dairy, separation is very unlikely)
- In young cattle (beef), separation might be a bit more likely as it is not that common to see very thin young beef cattle
- Condemnations under the disposition of emaciation usually occur during postmortem inspection rather than antemortem inspection
- Condemnation based on antemortem inspection might occur in animals severely emaciated (with or without diarrhoea) as these animals would be deemed to be of No Commercial Value, a food safety risk or a welfare concern, and sent to rendering.

The qualitative estimates were transformed into quantitative estimates using uniform distributions following the semi-quantitative methodology described at the Guidelines for Import Risk Analysis (DAFF, 2004).

3.5.3 Abattoir

As in the sheep assessment, the abattoir module for the cattle models is a representation of the process starting with a batch of animals entering the slaughter line and finishing with the different type of products originating from these animals. This module is based on a simulation model, with different batches of 50 animals coming from the lairage (five different scenarios, antemortem separation) going through the slaughter line. This model accounts for the potential cross-contamination among carcasses due to the amount of faeces present on the carcass surface. As such, each carcass has estimated quantities of internal MAP presence as well as external MAP contamination. The amount of internal MAP will depend on the animal level of infection. The external MAP contamination will be due to faeces coming from the intestines of the animal itself, which depends on the animal level of infection; and from faecal contamination of the hide, which depends on the level of infection of the animal and other animals in the batch. The parameters used for this simulation model are described in Probability of *disseminated infection*

Similarly than for sheep, not all infected cattle will have disseminated MAP infection in tissues other than the gastrointestinal tract, and therefore in the final product. Specific information on dissemination of infection among paucibacillary and multibacillary infected cattle is sparse, with only limited published studies with dissemination data. Reddacliff et al. (2010), among 9 adult cattle with clinical paratuberculosis, 1 (11.1%) and 5 (55.6%) animals had MAP in muscle (rump or forequarter) and peripheral lymph nodes (prescapular and/or prefemoral), respectively. Pribylova et al. (2011), in a study investigation the MAP counts in gastrointestinal tract, diaphragm and masseter of dairy cattle, reported 84.2% of intestine samples, 40 to 68% of diaphragms and 11.1 to 38.9% of masseters, with presence of MAP. Antognoli et al. (2008), among animals with mild gross lesions in the intestine (*normal appearing gastrointestinal tract and slight thickening of the ileum with obvious lymphatic involvement*), approximately between 33 to 37% had disseminated infection in the lymph nodes. This percentage increased to 63 to 100% in animals with more severe gross lesions (*moderate to severe thickened mucosa of the ileum and jejunum, enlargement of the associated mesenteric lymph nodes and pronounced thickening of serosal lymphatic vessels)*.

The current assessment estimated the probability of disseminated infection in the different products considered in this assessment from these previous studies as detailed in Table 13.

3.5.3.1 Amount of MAP in product

The amount of MAP in a serving size of each product considered in this assessment (100g for muscle and liver; 10g for intestines) was estimated for those animals with disseminated infection. As previously mentioned, there is limited specific information on the amount of MAP in product according to the animal disease pathology. As such, findings from the Reddacliff et al. (2010) study among nine cattle with clinical paratuberculosis, assumed to be multibacillary animals, were used. The animal with disseminated MAP in muscle had 58.9 MAP CFU/ g of tissue. Since there was only one animal, a Pert distribution adding 30% uncertainty to obtain the minimum and maximum values for the distribution was used. Among the five animals with disseminated infection in the lymph node, the mean MAP amount reported was 123.0 MAP CFU (3.1 d.s.) / g. This amount was incorporated into the model using a Normal distribution. No information was available on the amount of MAP in product for paucibacillary animals, as such, an extrapolation using the amount of MAP in product in multibacillary animals minus the difference between the amounts of MAP found in paucibacillary and multibacillary sheep (20 to 47% less MAP in paucibacillary compared to multibacillary) were used.

The amount of MAP in liver tissue was assumed to be the same as that in muscle, and the amount of MAP in intestines was assumed to be the same as that in faeces.

For skeletal (trim) and whole muscle (prime cut) and liver, the model accounts for the amount of MAP in tissue as well as the lymph nodes present in the tissue. As such, estimates on the amount of lymph nodes in each of these products were required. Since no information was available on this parameter, as in the sheep model, the cattle models used the following assumed parameters:

- Skeletal muscle (3 kg): Minimum, 1.5; Most likely, 3; Maximum, 6.
- Whole muscle (2 kg): Minimum, 0.5; Most likely, 1; Maximum, 2.
- Liver (4500 – 5000 grams): Minimum, 3; Most likely, 6; Maximum, 9.

These values were incorporated into the model using Pert distributions to account for uncertainty around these estimates, and the amount for a serving size of 100 grams was used.

Table 13. The first parameter (MAP presence by consignment type) is the output from the lairage model. A detailed description of the rest of the nodes follows.

The output of this module is the average amount of MAP (in Log10) in a serving size of the product for each animal within a consignment. For each scenario (prevalence area, beef vs. dairy), different products were considered. As previously described, these products for cattle are: skeletal muscle (trim), whole muscle (prime cut), liver and intestines.

3.5.3.2 Transfer of external MAP to carcass surface

This parameter accounts for: the probability of faecal matter being transferred onto the carcass surface; the grams of faeces being transferred; the proportion of faeces originating from hide and intestines; and, the amount of MAP present in faeces. The expert opinion elicited during the consultation process with the Scientific Risk Management Panel of the Food Safety program within Meat and Livestock Australia (*Section 3.3.2.2., d*) was used as the source to estimate the required inputs for this parameter. The probability of transfer of faecal matter onto the carcass surface and the proportion of faeces originating from hide and intestines has been previously explained in the sheep assessment (*Section 3.4.3.1*), and the same estimates were used.

Grams of faeces transferred: According to the output of the expert elicitation exercise, the grams of faeces present on the carcass surface of adult and young cattle were defined by the following distributions (median, 5-95%):

- Adult cattle carcasses: 0.769 (0.076 – 5.204)
- Young cattle carcasses: 0.708 (0.062 – 5.083)

Amount of MAP in faeces: This parameter estimates the number of MAP (Colony-forming unit, CFU) per gram of faeces of infected animals. Only those animals with paucibacillary and multibacillary disease pathology were considered to be shedding MAP in faeces. Limited studies are available with information of MAP shedding according to cattle disease pathology. A study by Pribylova et al (2011), using 25 cows with different clinical status of MAP infection from three dairy herds, was used to estimate the amount of MAP in faeces of Johne's disease infected cattle. According to this study, 64.0% of the animals were low to moderate shedders (10^2 – 10^4) and 24.0% were high to heavy shedders (≥10^4). This assessment considered paucibacillary infection in those animals with low to moderate shedding and multibacillary infection in those high to heavy shedders. Among paucibacillary animals, the median shedding was 838.5 CFU of MAP per gram of faeces (min, 191; max, 6,280); while among multibacillary animals, the median shedding was 62,850 CFU MAP per gram of faeces (min, 10,000; max, 9.6×10^5). A Pert distribution was used to incorporate these parameters into the model.

3.5.3.3 Probability of disseminated infection

Similarly than for sheep, not all infected cattle will have disseminated MAP infection in tissues other than the gastrointestinal tract, and therefore in the final product. Specific information on dissemination of infection among paucibacillary and multibacillary infected cattle is sparse, with only limited published studies with dissemination data. Reddacliff et al. (2010), among 9 adult cattle with clinical paratuberculosis, 1 (11.1%) and 5 (55.6%) animals had MAP in muscle (rump or forequarter) and peripheral lymph nodes (prescapular and/or prefemoral), respectively. Pribylova et al. (2011), in a study investigation the MAP counts in gastrointestinal tract, diaphragm and masseter of dairy cattle, reported 84.2% of intestine samples, 40 to 68% of diaphragms and 11.1 to 38.9% of masseters, with presence of MAP. Antognoli et al. (2008), among animals with mild gross lesions in the intestine (*normal appearing gastrointestinal tract and slight thickening of the ileum with obvious lymphatic involvement*), approximately between 33 to 37% had disseminated infection in the lymph nodes. This percentage increased to 63 to 100% in animals with more severe gross lesions (*moderate to severe thickened mucosa of the ileum and jejunum, enlargement of the associated mesenteric lymph nodes and pronounced thickening of serosal lymphatic vessels)*.

The current assessment estimated the probability of disseminated infection in the different products considered in this assessment from these previous studies as detailed in Table 13.

3.5.3.4 Amount of MAP in product

The amount of MAP in a serving size of each product considered in this assessment (100g for muscle and liver; 10g for intestines) was estimated for those animals with disseminated infection. As previously mentioned, there is limited specific information on the amount of MAP in product according to the animal disease pathology. As such, findings from the Reddacliff et al. (2010) study among nine cattle with clinical paratuberculosis, assumed to be multibacillary animals, were used. The animal with disseminated MAP in muscle had 58.9 MAP CFU/ g of tissue. Since there was only one animal, a Pert distribution adding 30% uncertainty to obtain the minimum and maximum values for the distribution was used. Among the five animals with disseminated infection in the lymph node, the mean MAP amount reported was 123.0 MAP CFU (3.1 d.s.) / g. This amount was incorporated into the model using a Normal distribution. No information was available on the amount of MAP in product for paucibacillary animals, as such, an extrapolation using the amount of MAP in product in multibacillary animals minus the difference between the amounts of MAP found in

paucibacillary and multibacillary sheep (20 to 47% less MAP in paucibacillary compared to multibacillary) were used.

The amount of MAP in liver tissue was assumed to be the same as that in muscle, and the amount of MAP in intestines was assumed to be the same as that in faeces.

For skeletal (trim) and whole muscle (prime cut) and liver, the model accounts for the amount of MAP in tissue as well as the lymph nodes present in the tissue. As such, estimates on the amount of lymph nodes in each of these products were required. Since no information was available on this parameter, as in the sheep model, the cattle models used the following assumed parameters:

- Skeletal muscle (3 kg): Minimum, 1.5; Most likely, 3; Maximum, 6.
- Whole muscle (2 kg): Minimum, 0.5; Most likely, 1; Maximum, 2.
- Liver (4500 – 5000 grams): Minimum, 3; Most likely, 6; Maximum, 9.

These values were incorporated into the model using Pert distributions to account for uncertainty around these estimates, and the amount for a serving size of 100 grams was used.

Table 13. Input parameter and input values used for the slaughter floor simulation component of the exposure of *Mycobacterium avium paratuberculosis* (MAP) to human in cattle

Node	Parameter estimates	Input value	Data sources
1. MAP presence by consignment type	Proportion of cattle within a consignment in each level of MAP infection	-	Output from the lairage scenario
2. Transfer of external MAP to carcass surface	Probability of transfer of faecal matter onto the carcass surface	Point estimate = 1	ESAM data Expert opinion
	Grams of faeces transferred Proportion of faeces from hide and intestines	Individual expert opinion: Pert (lowest, most likely, highest) Combined expert opinion: Discrete (Outcome Pert, expert opinion weights) Output Discrete (median, 5-95%)	
	CFU MAP Log10 / g of faeces	Paucibacillary: Pert(2.3,2.9,3,8) Multibacillary: Pert(4.0,4.8,6.0)	Pribylova et al. (2011)
3. Probability of disseminated infection	Probability of infected animals having a disseminated infection in muscle, lymph nodes, liver and intestines (different according to disease pathology)	Paucibacillary: Muscle, Liver, Beta(2,9); Lymph node, Uniform(0.33,0.38); Intestines, 1. Multibacillary: Muscle, Liver, Uniform(0.40,0.68); Lymph node, Uniform(0.64,1.0); Intestines, 1.	Antognoli et al. (2008); Prybilova et al. (2011); Reddacliff et al. (2010)
4. Amount of MAP in product	CFU MAP Log10/ g of product (lymph node, muscle, liver, intestines)	Paucibacillary: Muscle, Liver, Lymph nodes (Multibacillary estimates minus 20-47%); Intestines (as faeces) Multibacillary: Muscle, Liver, Pert(1.6,1.8,1.9); Lymph node, Normal(2.09,0.49); Intestines (as faeces)	Pribylova et al. (2011); Reddacliff et al. (2010)

3.5.4 Sensitivity analysis

Following the same methodology than for the sheep exposure assessment, the sensitivity of the some of the outputs of the model to some of the input parameters was investigated using the @Risk Advanced Sensitivity Analysis (@RISK 6.0, Palisade Corporation, USA). The influence of each input parameter on the model outputs was evaluated by simulating the outputs using a series of fixed values for a given input variable. Some input parameters evaluated were incorporated into the analyses due to the significant uncertainty around the input values used in the model. Within the scenario with the highest herd prevalence (S4), the following input parameters were investigated: herd prevalence (P_H), within-herd prevalence (P_U), probability of showing clinical signs (*Prob_CS*), probability of sending multibacillary animals to the abattoir (related to the disease pathology node of the on-farm scenario) and the probability of antemortem detection (*ProbAM*). The outputs monitored were the probability of animals with high MAP infection being sent to the abattoir, and entering the slaughter line. Moreover, the influence of these parameters on the amount of MAP in final product was also investigated in addition to that on the amount of grams of faeces.

Proportion and probability input parameters were allowed to vary from 0 to 1 in tenths (0.1, 0.2, 0.3...). Each of the values for each input parameter was evaluated separately in a simulation of 5,000 iterations, whilst values for all other input variables were fixed to the base value.

4 Results

4.1 Exposure assessment for the sheep industry

4.1.1 On-farm

Table 14 shows the model outputs (median, 5 – 95%) for the probability of sheep within different MAP infection categories and within each geographical area and animal type (adult animals and young animals) being sent to the abattoir. These categories were defined as:

- Non-infected animals from infected flocks
- Animals with low level of MAP infection (Paucibacillary animals) from infected flocks
- Animals with high level of MAP infection (Multibacillary animals) from infected flocks
- Non-infected animals from non-infected flocks

The highest probability of sending infected animals (low and high level of MAP infection) to the abattoir is of batches from geographical area 2 and adult animals. The probabilities of sending animals with high levels of MAP from a high prevalence region (GA 2) are 5 per 10,000 adult sheep and 2 per 10,000 lambs. Within infected flocks, these probabilities increase to 16 per 10,000 adult sheep and 5 per 10,000 lambs. The probability of infected animals to be sent to slaughter from low or extremely low Ovine Johne's disease prevalence regions is much lower (Table 14). As previously mentioned, since the animal-level post-vaccination prevalence has been estimated based on shedding information, the probability of infected animals being sent to the abattoir might be underestimated.

For example, considering batches of adult sheep originating from GA2, 70.3% (54.5% – 81.6%) of batches would originate from non-infected flocks and as such do not pose a risk of MAP exposure to humans; with the rest (29.7%) coming from infected flocks. However, this assessment indicates that among animals within these infected flocks, most (99.6%, 99.0% - 99.8%) would be non-infected, 0.25% (0.08% - 0.67%) would have low level of MAP (Paucibacillary) and 0.16% (0.05% - 0.41%) would have high level of MAP (Multibacillary). An alternative interpretation would be that among all adult sheep from direct consignments from GA2, 70.3% (54.5% – 81.6%) of the animals would be non-infected from non-infected flocks, 29.4% (18.2% - 45.1%) non-infected animals from infected flocks, 0.07% (0.02% - 0.22%) infected animals with low level of MAP and 0.05% (0.01% - 0.13%) infected animals with high level of MAP.

Table 14. Predicted probability of sheep within various categories of MAP infection are sent to the abattoir. Predictions are presented by geographical area* and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Geographical area / Animal Type	Probability			
	Among all animals		Among animals within infected flocks	
	Median	5% - 95%	Median	5% - 95%
GA1 Adult sheep				
Non-infected / Infected flocks	0.0066	(0.0032- 0.0186)	0.9895	(0.9758 - 0.9963)
Low MAP (Paucibacillary)	4.0×10^{-5}	(1.0×10^{-5} - 0.0001)	0.0060	(0.0019 - 0.0161)
High MAP (Multibacillary)	2.7×10^{-5}	(6.9×10^{-6} -8.2×10^{-5})	0.0040	(0.0012 - 0.0096)
Non-infected / Non-infected flocks	0.9933	(0.9877 - 0.9968)		
GA2 Young sheep				
Non-infected / Infected flocks	0.2990	(0.1832 - 0.4524)	0.9980	(0.9950 - 0.9993)
Low MAP (Paucibacillary)	0.0004	(0.0001 - 0.0014)	0.0015	(0.0004 - 0.0041)
High MAP (Multibacillary)	0.0002	(3.7×10^{-5} - 0.0005)	0.0005	(0.0001 - 0.0016)
Non-infected / Non-infected flocks	0.7029	(0.5450 - 0.8160)		
GA2 Adult Sheep				
Non-infected / Infected flocks	0.2941	(0.1823 - 0.4506)	0.9958	(0.9897 - 0.9984)
Low MAP (Paucibacillary)	0.0007	(0.0002 - 0.0022)	0.0025	(0.0008 - 0.0067)
High MAP (Multibacillary)	0.0005	(0.0001 - 0.0013)	0.0016	(0.0005 - 0.0041)
Non-infected / Non-infected flocks	0.7029	(0.5450 - 0.8160)		
GA3 Young sheep				
Non-infected / Infected flocks	0.0443	(0.0295 - 0.0677)	0.9953	(0.9880 - 0.9986)
Low MAP (Paucibacillary)	0.0001	(3.4×10^{-5} -0.0004)	0.0032	(0.0008 - 0.0090)
High MAP (Multibacillary)	5.2×10^{-5}	(1.2×10^{-5} -0.0002)	0.0012	(0.0003 - 0.0037)
Non-infected / Non-infected flocks	0.9551	(0.9316-0.9703)		
GA3 Adult Sheep				
Non-infected / Infected flocks	0.0437	(0.02890-0.06673)	0.9896	(0.9760 - 0.9964)
Low MAP (Paucibacillary)	0.0003	(8.0×10^{-5} -0.0008)	0.0060	(0.0019 - 0.0161)
High MAP (Multibacillary)	0.0002	(5.0×10^{-5} -0.0005)	0.0039	(0.0012 - 0.0096)
Non-infected / Non-infected flocks	0.9551	(0.9316-0.9703)		

*GA1, Geographical Area 1 – Extremely low Ovine Johne's disease prevalence; GA2, Geographical Area 2, High Ovine Johne's disease prevalence; GA3, Geographical Area 3, Low Ovine Johne's disease prevalence

4.1.2 Lairage

For all geographical areas and animal types, within infected consignments, the majority of animals entering the slaughter line have no MAP internally (non-infected animals and animals with disease pathology Perez 1 & 2) and low MAP externally due to faecal cross-contamination from other animals in the batch. Thus, the majority of carcasses and therefore product will originate from animals with only low level of MAP on the carcasses surface with a *very* to *extremely low* proportion of animals with internal infection and high level of MAP externally.

For those animals with low to high level of internal infection (Paucibacillary and Multibacillary animals) arriving at the abattoir, the probability of clinically affected animals being separated during antemortem inspection activities is very low and as such, the proportion of animals within this category is *extremely low* to *negligible*. Similarly, the proportion of Johne's disease infected animals being separated and condemned is *extremely low* to *negligible*. The reasons are the low probability of infected animals showing clinical signs and the very low probability of these animals being separated during antemortem inspection. Therefore, most animals with low to high level of internal infection will enter the slaughter floor and be used for human consumption, posing a risk for human exposure.

Within the main lots, the slightly higher probability of low and high MAP internal and external in consignments of adult sheep from GA1 and GA3 than in consignments of adult sheep from GA2 is due to the fact that within infected flocks (which are the only ones considered in Table 15), the probability of vaccination is higher in GA2 than in the other areas, having an effect on the individual prevalence as well as the disease pathology distribution among infected animals.

Table 15 shows the model outputs (median, 5% - 95%) for the lairage module, with only considering infected consignments within each geographical area and animal type (adult animals and young animals). The output categories defined for the lairage module as previously described were:

- Non-infected animals from non-infected flocks (the same as for the on-farm output)
- Infected flocks:
    - Main lot:
        - Animals with no MAP infection (non infected and Perez 1 or 2) and low levels of external MAP
        - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
        - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
    - Separated lot (during antemortem inspection activities):
        - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
        - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
        - Condemned animals

For all geographical areas and animal types, within infected consignments, the majority of animals entering the slaughter line have no MAP internally (non-infected animals and animals with disease pathology Perez 1 & 2) and low MAP externally due to faecal cross-contamination from other animals in the batch. Thus, the majority of carcasses and therefore product will originate from animals with only low level of MAP on the carcasses surface with a *very* to *extremely low* proportion of animals with internal infection and high level of MAP externally.

For those animals with low to high level of internal infection (Paucibacillary and Multibacillary animals) arriving at the abattoir, the probability of clinically affected animals being separated during antemortem inspection activities is very low and as such, the proportion of animals within this category is *extremely low* to *negligible*. Similarly, the proportion of Johne's disease infected animals being separated and condemned is *extremely low* to *negligible*. The reasons are the low probability of infected animals showing clinical signs and the very low probability of these animals being separated during antemortem inspection. Therefore, most animals with low to high level of internal infection will enter the slaughter floor and be used for human consumption, posing a risk for human exposure.

Within the main lots, the slightly higher probability of low and high MAP internal and external in consignments of adult sheep from GA1 and GA3 than in consignments of adult sheep from GA2 is due to the fact that within infected flocks (which are the only ones considered in Table 15), the probability of vaccination is higher in GA2 than in the other areas, having an effect on the individual prevalence as well as the disease pathology distribution among infected animals.

Table 15. Predicted probability of sheep within various categories of MAP infection, within infected flocks, enter the slaughter line from the lairage. Predictions are presented by geographical area*, animal type and consignment type† and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Geographical area / Animal Type / Consignment type	**Probability**	
	Among animals from infected flocks	
	Median	5% - 95%
GA1 Adult sheep		
Main lot		
No MAP internal / Low MAP External	0.9895	(0.9758 - 0.9963)
Low MAP internal / High MAP external	0.0060	(0.0019 - 0.0161)
High MAP	0.0040	(0.0012 – 0.0096)
Separated lot		
Low MAP internal / High MAP external	1.6×10^{-6}	(1.2×10^{-7} – 9.8×10^{-6})
High MAP	7.5×10^{-6}	(8.0×10^{-7} - 2.8×10^{-5})
Condemned	1.9×10^{-6}	(1.9×10^{-7} - 9.1×10^{-6})
GA2 Young sheep		
Main lot		
No MAP internal / Low MAP External	0.9981	(0.9955 – 0.9993)
Low MAP internal / High MAP external	0.0012	(0.0004 – 0.0033)
High MAP	0.0005	(0.0001- 0.0014)
Separated lot		
Low MAP internal / High MAP external	3.1×10^{-7}	(2.1×10^{-8} – 1.8×10^{-6})
High MAP	7.7×10^{-7}	(8.0×10^{-8} – 3.7×10^{-6})
Condemned	5.2×10^{-5}	(4.6×10^{-6} – 0.0003)
GA2 Adult Sheep		
Main lot		
No MAP internal / Low MAP External	0.9962	(0.9916 – 0.9984)
Low MAP internal / High MAP external	0.0022	(0.0008 – 0.0054)
High MAP	0.0014	(0.0005 – 0.0032)
Separated lot		
Low MAP internal / High MAP external	5.5×10^{-7}	(4.4×10^{-8} – 3.1×10^{-6})
High MAP	2.4×10^{-6}	(3.0×10^{-7} – 9.5×10^{-6})
Condemned	5.2×10^{-5}	(4.7×10^{-6} – 0.0003)
GA3 Young sheep		
Main lot		
No MAP internal / Low MAP External	0.9954	(0.9881 – 0.9985)
Low MAP internal / High MAP external	0.0031	(0.0009 – 0.0094)
High MAP	0.0011	(0.0003 – 0.0038)
Separated lot		
Low MAP internal / High MAP external	8.8×10^{-7}	(5.7×10^{-8} – 5.4×10^{-6})
High MAP	2.1×10^{-6}	(2.1×10^{-7} – 1.0×10^{-5})
Condemned	5.2×10^{-5}	(4.9×10^{-6} – 0.0003)
GA3 Adult Sheep		
Main lot		
No MAP internal / Low MAP External	0.9896	(0.9763 – 0.9963)
Low MAP internal / High MAP external	0.0060	(0.0019 – 0.0157)
High MAP	0.0040	(0.0013 – 0.0095)
Separated lot		
Low MAP internal / High MAP external	1.6×10^{-6}	(1.2×10^{-7} – 9.7×10^{-6})
High MAP	7.4×10^{-6}	(8.0×10^{-7} – 2.8×10^{-5})
Condemned	5.4×10^{-5}	(5.3×10^{-6} –0.0003)

*GA1, Geographical Area 1 – Extremely low Ovine Johne's disease prevalence; GA2, Geographical Area 2, High Ovine Johne's disease prevalence; GA3, Geographical Area 3, Low Ovine Johne's disease prevalence.† Separated lot = Lot of animals clinically affected with Johne's disease, detected and separated during antemortem inspections at lairage.

4.1.3 Abattoir

For infected consignments and the different lots coming from the lairage,

Table **16** shows the predicted median amount of MAP (Log10 CFU) in a serving size of product for an average animal of each consignment. The average amount of MAP in an animal from these consignments is very similar, because the proportion of animals within each of the disease category, as described in the lairage module, is very similar within infected consignments, independently of the geographical area.

Among main lots from infected flocks (lots with non infected, Perez 1&2, paucibacillary and multibacillary animals), the amount of MAP in skeletal muscle from an adult sheep coming from GA2, is estimated to be 0.45 (0.34 – 3.80) Log CFU/100 g; and this amount is similar for 100g of whole muscle from young sheep from the same area (0.48; 0.39 – 3.78 Log CFU/100g). Liver produced from young sheep from GA2, has a median amount of MAP of 0 (0 to -0.60) Log CFU/100 g, indicating that this product poses an extremely low to negligible risk of MAP exposure to humans. Regarding intestines from adult sheep from GA2, although the median amount of MAP per 10 g of product is 0 Log CFU, since most animals in an infected consignment will not be infected and will only have MAP on the carcass surface as previously explained; the 95 percentile of the output distribution is 7.30 Log CFU, representing those infected animals.

Those consignments coming from separated animals during antemortem inspection entering the slaughter line, which are infected animals with low and high level of infection (Paucibacillary and Multibacillary animals only), have the highest amount of MAP in final product. For example, skeletal muscle from adult sheep from GA2 have an estimated amount of MAP of 5.43 (5.33 – 5.60) Log CFU / 100g and the amount of MAP in intestines is estimated higher (8.90; 8.66 – 8.99 Log CFU/ 10g). However, as shown in the lairage module, the probability of these animals entering the slaughter line in a separated lot is *extremely low* to *negligible* and therefore, most of these animals will enter the slaughter line with the main lot.

Table 16. Predicted amount of MAP (Log10 CFU) in a serving size of product for an average animal within an infected consignment. Predictions are presented by geographical area*, animal type, consignment type‡ and animal product, and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Geographical area / Animal Type / Consignment type / Product	**Total MAP Log CFU in a serving size† of product**		
	Median	5%	95%
GA1 Adult sheep			
Main lot Skeletal Muscle	1.24	0.39	4.16
Separated Skeletal Muscle	5.52	5.33	5.61
GA2 Young sheep			
Main lot Whole Muscle	0.48	0.39	3.78
Separated Whole Muscle	5.47	5.38	5.65
Main lot Liver	0.00	0.00	-0.60
Separated lot Liver	3.74	3.42	3.93
GA2 Adult Sheep			
Main lot Skeletal Muscle	0.45	0.34	3.80
Separated Skeletal Muscle	5.43	5.33	5.60
Main lot Intestines	0.00	0.00	7.30
Separated Intestines	8.90	8.66	8.99
GA3 Young sheep			
Main lot Whole Muscle	0.50	0.39	3.93
Separated Whole Muscle	5.47	5.37	5.66
GA3 Adult Sheep			
Main lot Skeletal Muscle	1.22	0.36	4.05
Separated Skeletal Muscle	5.43	5.33	5.61
Main lot Intestines	0.00	0.00	7.30
Separated Intestines	8.90	8.66	8.99

*GA1, Geographical Area 1 – Extremely low Ovine Johne's disease prevalence; GA2, Geographical Area 2, High Ovine Johne's disease prevalence; GA3, Geographical Area 3, Low Ovine Johne's disease prevalence; †Serving size: 100 g muscle and liver, 10g of intestines; ‡ Separated lot = Lot of animals clinically affected with Johne's disease, detected and separated during antemortem inspections at lairage.

4.1.4 Overall probability of exposure

Considering the entire production chain from the farm to the product, Table 17 presents the overall probability of animals with the different disease pathology categories entering the slaughter line among all animals coming from each geographical area (infected and non-infected flocks). This table shows that most animals coming from GA1 (99.3%; 98.8 – 99.7%) and GA3 (95.5%; 93.2 – 97.0%) will be non-infected animals from non-infected flocks, and as such, posing a negligible to no risk of MAP exposure to humans. The rest of the animals from GA1, are mainly animals with no infection (No MAP internal) and low MAP external due to cross-contamination in lairage (0.65%; 0.3% - 1.2%). An *extremely low* to

negligible proportion of animals will be infected (low or high MAP) with high MAP contamination on the carcass surface. Similar results are estimated in animals from GA3, with most of the rest of the animals being animals with no infection (No MAP internal) and low MAP external due to cross-contamination in lairage (4.5%; 2.9% - 6.7%), with an *extremely low* to *negligible* proportion of animals being infected (low or high MAP) with high MAP contamination on the carcass surface.

In contrast, among animals from GA2, due to the higher flock prevalence in this area, the proportion of non-infected animals from non-infected flocks is estimated 70.0% (54.8% - 81.4%), which is lower than in the other areas. Therefore, as expected, the overall risk of human exposure to MAP is higher among animals from GA2 than the other areas. However, the probability of exposure is still low, when considering that among the rest of the animals from GA2, most are non-infected animals (No MAP internal) with only low MAP on the carcass surface (29.9%; 18.4% - 45.1%). An *extremely low* to *negligible* proportion of animals from GA2 will be infected (low or high MAP) with high MAP external contamination.

Table 17. Predicted probability of sheep within different MAP infection categories enter the slaughter line. Predictions are presented by geographical area*, animal type and consignment type, and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Geographical area / Animal Type / Consignment type	**Probability**	
	Among animals from the geographical area	
	Median	5 %– 95%
GA1 Adult sheep		
No MAP - Non Infected flocks	0.9933	(0.9880 – 0.9967)
No MAP internal / Low MAP external	0.0065	(0.0032 – 0.0119)
Low MAP internal / High MAP external	3.9×10^{-5}	$(9.9 \times 10^{-6} – 0.0001)$
High MAP	2.7×10^{-5}	$(6.9 \times 10^{-6} – 8.2 \times 10^{-5})$
No food chain	1.2×10^{-8}	$(1.2 \times 10^{-9} – 6.5 \times 10^{-8})$
GA2 Young sheep		
No MAP - Non Infected flocks	0.7001	(0.5481 – 0.8143)
No MAP internal / Low MAP external	0.2985	(0.1840 – 0.4511)
Low MAP internal / High MAP external	0.0004	$(1.1 \times 10^{4} – 0.001)$
High MAP	0.0001	$(3.8 \times 10^{-5} – 4.0 \times 10^{-4})$
No food chain	1.6×10^{-5}	$(1.4\times 10^{-6} – 9.0 \times 10^{-5})$
GA2 Adult Sheep		
No MAP - Non Infected flocks	0.7001	(0.5481 – 0.8143)
No MAP internal / Low MAP external	0.2985	(0.1840 – 0.4510)
Low MAP internal / High MAP external	0.0006	(0.0002 – 0.0016)
High MAP	0.0004	$(1.4 \times 10^{-4} – 0.0010)$
No food chain	1.6×10^{-5}	$(1.4 \times 10^{-6} – 9.0 \times 10^{-5})$
GA3 Young sheep		
No MAP - Non Infected flocks	0.9546	(0.9322 – 0.9704)
No MAP internal / Low MAP external	0.0451	(0.0295 – 0.0672)
Low MAP internal / High MAP external	0.0002	$(3.7 \times 10^{-5} – 0.0005)$
High MAP	5.1×10^{-5}	$(1.3 \times 10^{-5} – 1.9 \times 10^{-4})$
No food chain	2.4×10^{-6}	$(2.0 \times 10^{-7} – 1.4 \times 10^{-5})$
GA3 Adult Sheep		
No MAP - Non Infected flocks	0.9546	(0.9322 – 0.9704)
No MAP internal / Low MAP external	0.0449	(0.0291 – 0.0667)
Low MAP internal / High MAP external	0.0003	$(7.6 \times 10^{-5} – 0.0008)$
High MAP	0.0002	$(5.3 \times 10^{-5} – 0.0005)$
No food chain	2.4×10^{-6}	$(2.0 \times 10^{-7} – 1.4 \times 10^{-5})$

*GA1, Geographical Area 1 – Extremely low Ovine Johne's disease prevalence; GA2, Geographical Area 2, High Ovine Johne's disease prevalence; GA3, Geographical Area 3, Low Ovine Johne's disease prevalence

4.1.5 Sensitivity analysis

The influence of flock vaccination (*Pr_FlockVacc*), probability of sending multibacillary animals to the abattoir (related to the disease pathology node of the on-farm scenario) and the probability of antemortem detection (*ProbAM*) on the probability of infected animals with high level of MAP being sent to the abattoir and entering the slaughter line is shown in

Figure 6. The level of flock vaccination is the input variable with most influence on the output of the model. When the flock vaccination increases to 100% among infected flocks, a 3-fold decrease is recorded in the probability of high MAP animals entering the slaughter line. Most importantly, if flock vaccination in this area (which is currently estimated to be approximately 70%) is reduced to 10% the probability of high MAP animals entering the slaughter line would increase 3 times. This suggests that maintaining high vaccination coverage among infected flocks is important for reducing the probability of exposure of MAP to humans.

Similarly, when the probability of sending multibacillary animals to the abattoir decreases to 0.2, the probability of sending high MAP animals would decrease by half; suggesting that awareness and attitudes of producers regarding sending animals with Johne's disease clinical signs are important in reducing the risk of MAP exposure to humans. The effect of the probability of antemortem inspection activities detecting and separating clinically affected animals is very limited as only those animals showing clinical signs (approximately 1% and 10% of paucibacillary and multibacillary animals, respectively) are able to be detected and separated.

The influence of the proportion of animals vaccinated within vaccinated flocks (*Pr_WFVacc*) on the probability of animals with high MAP infection entering the slaughter line among young sheep from GA2 was also monitored. The sensitivity results indicated that even when all young sheep are vaccinated the probability of animals with high MAP infection entering the slaughter line only decreases from a median of 0.0005 to 0.0004, suggesting that there

would be very limited effect on vaccinating young animals to be sent to the abattoir at this age. The main explanation for this limited effect is that the proportion of multibacillary animals within young sheep is very low independently of vaccination status.

The effect of the flock prevalence on the proportion of high MAP animals entering the slaughter line was investigated in adult sheep consignments from GA3. In this area the flock prevalence is estimated to be between 2 to 5%. When the prevalence increases to 10%, there is a 2.2-fold increase in the proportion of animals with high MAP infection entering the slaughter line. This increase is 6.7-fold when the prevalence is increased to 30%. This supports the importance of maintaining biosecurity and management practices to avoid the spread of disease between properties and from geographical areas with high prevalence.

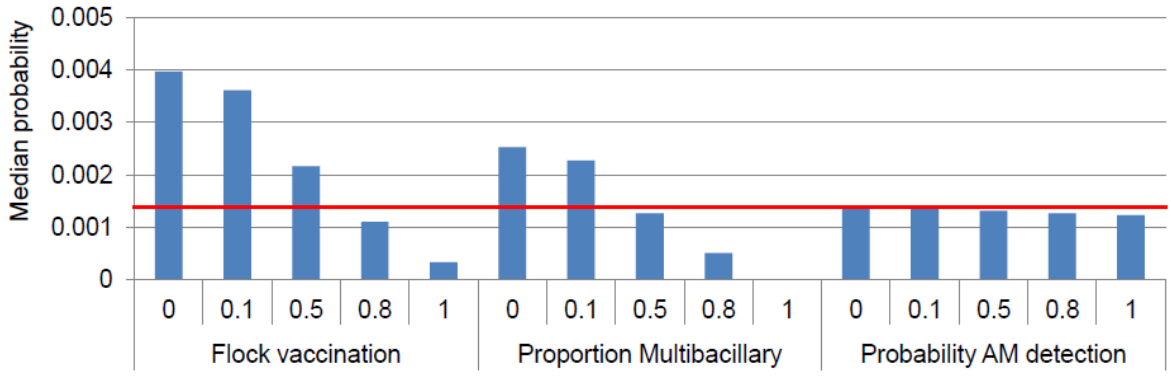

Figure 6. Sensitivity analysis depicting the influence of key input variables on the median probability (solid horizontal line) of infected animals with high level of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) entering the slaughter line at the abattoir. Key input variables evaluated: a) proportion of flocks adopting vaccination, b) probability of multibacillary-diseased animals being sent for slaughter and c) probability of detection of MAP clinical cases at ante-mortem inspection. Height of bars represents the median probability of infected animals with high level of MAP entering the slaughter line at the abattoir according to each value used for the specific input variable. Results were obtained from a simulation of 5,000 iterations using @Risk's Advanced Sensitivity Analysis.

The influence of the same input parameters (*Pr_FlockVacc*, probability of sending multibacillary animals to the abattoir and *ProbAM*) on the amount of MAP in final product was also investigated in addition to the grams of faeces present the carcass. As shown in

Figure **7**, the amount of MAP in product from animals from infected consignments would increase 5-fold if there were no flocks being vaccinated. However, the most significant effect

on this output is due to a variation of the grams of faeces in the carcass, as expected, indicating the importance of maintaining good hygienic practices at the abattoir to reduce carcass contamination with faeces.

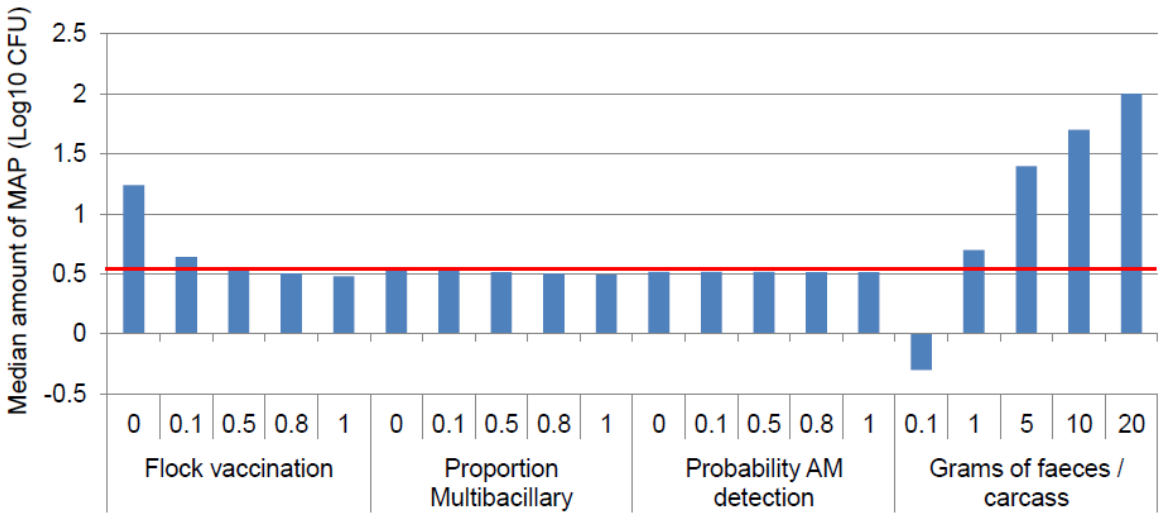

Figure 7. Sensitivity analysis depicting the influence of key input variables on the median amount (solid horizontal line) of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) CFU (Log10) in 100g of skeletal muscle of an adult sheep from an infected consignment originating from geographical area 2 (high flock prevalence) considered in this assessment. Key input variables evaluated: a) proportion of flocks adopting vaccination, b) probability of multibacillary-diseased animals being sent for slaughter, c) probability of detection of MAP clinical cases at ante-mortem inspection and d) Grams of faeces present on the carcass surface. Height of bars represents the median amount of MAP according to each value used for the specific input variable. Results were obtained from a simulation of 5,000 iterations using @Risk's Advanced Sensitivity Analysis.

4.2 Exposure assessment for the cattle industry

4.2.1 On-farm

Table **18** shows the model outputs (median, 5 – 95%) for the probability of cattle within different MAP infection categories and within each scenario considered in this assessment being sent to the abattoir. These categories were defined as:

- Non-infected animals from infected herds
- Animals with low level of MAP infection (Paucibacillary animals) from infected herds
- Animals with high level of MAP infection (Multibacillary animals) from infected herds
- Non-infected animals from non-infected herds

The probability of sending infected beef cattle from S1 and S2 (low and high level of MAP infection) to the abattoir is *extremely low* to *negligible*, mainly due to the extremely low herd prevalence in these areas. As expected, the highest probability of sending infected animals to the abattoir is among dairy cattle sources from Scenario 4 (S4, Management areas) as this is the area with the highest herd prevalence. Among dairy adult cattle consignments originating at S4, 79.8% (78.9% – 80.7%) would come from non-infected herds, with the rest of consignments originating from infected herds. However, among the infected consignments most animals (99.6%; 99.3% - 99.8%) are non-infected animals, 0.07% (0.03%– 0.17%) would have low level of MAP and 0.33% (0.18% - 0.54%) would have high level of MAP. Among consignments of dairy cattle from Beef Protected areas (S3), 88.4% (86.8% - 89.9%) would be from non-infected herds. Similarly than for S4, most animals within infected consignments would not be infected, posing a very low risk of MAP exposure to humans. Most consignments from dairy cattle from the Protected zone (S5) would originate from non-infected herds (99.9%; 99.6 – 99.9%). As previously mention, these results should be interpreted with caution, since the available data on herd-prevalence is likely to underestimate the true herd-prevalence, and as such, the probability of sending infected animals to the abattoir might be higher to the probability estimated by this model.

Table 18. Predicted probability of cattle within various categories of MAP infection are sent to the abattoir. Predictions are presented by scenario* and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Scenario	Probability			
	Among all animals		Among animals within infected herds	
	Median	5% - 95%	Median	5% - 95%
S1 Beef				
Non-infected / Infected herds	2.50×10^{-5}	(1.8×10^{-5} - $1.1 \times 10^{-4)}$	0.9997	(0.9991 – 0.999)
Low MAP (Paucibacillary)	1.14×10^{-9}	(5.5×10^{-11} - 7.8×10^{-9})	5.0×10^{-5}	(1.0×10^{-5} – 0.0002)
High MAP (Multibacillary)	5.51×10^{-9}	(2.7×10^{-10} – 3.7×10^{-8})	0.0002	(5.0×10^{-5} - 0.0007)
Non-infected / Non-infected herds	0.9999	0.9999		
S2 Beef				
Non-infected / Infected herds	0.0010	(0.0008 – 0.0013)	0.9997	(0.9991 – 0.999)
Low MAP (Paucibacillary)	5.2×10^{-8}	(9.8×10^{-9} – 1.6×10^{-7})	5.0×10^{-5}	(1.0×10^{-5} – 0.0002)
High MAP (Multibacillary)	2.5×10^{-7}	(4.8×10^{-8} – 7.6×10^{-7})	0.0002	(5.0×10^{-5} - 0.0007)
Non-infected / Non-infected herds	0.9989	(0.9987- 0.9992)		
S3 Dairy				
Non-infected / Infected herds	0.1156	(0.1008 – 0.1314)	0.9960	(0.9933 – 0.9977)
Low MAP (Paucibacillary)	9.0×10^{-5}	(3.0×10^{-5} – 0.0002)	0.0007	(0.0003 – 0.0017)
High MAP (Multibacillary)	0.0004	(0.0002 – 0.0007)	0.0033	(0.0018 – 0.0054)
Non-infected / Non-infected herds	0.8839	(0.8680 – 0.8987)		
S4 Dairy				
Non-infected / Infected herds	0.2010	(0.1918 – 0.2104)	0.9960	(0.9933 – 0.9977)
Low MAP (Paucibacillary)	0.0002	(5.10×10^{-5} – 0.0004)	0.0007	(0.0003 – 0.0017)
High MAP (Multibacillary)	0.0007	(0.0004 – 0.0011)	0.0033	(0.0018 – 0.0054)
Non-infected / Non-infected herds	0.7981	(0.7887 – 0.8073)		
S5 Dairy				
Non-infected / Infected herds	0.0010	(7.6×10^{-5} – 0.0044)	0.9960	(0.9933 – 0.9977)
Low MAP (Paucibacillary)	7.2×10^{-7}	(4.7×10^{-8} – 4.2×10^{-6})	0.0007	(0.0003 – 0.0017)
High MAP (Multibacillary)	3.3×10^{-6}	(2.3×10^{-7} – 1.6×10^{-5})	0.0033	(0.0018 – 0.0054)
Non-infected / Non-infected herds	0.9989	(0.9955 – 0.9999)		

S1, Beef, Protected and Free zones; S2, Beef, Beef Protected and Management areas; S3, Dairy, Beef Protected area; S4, Dairy, Management area; S5, Dairy, Protected zone)

4.2.2 Lairage

The model outputs (median, 5% - 95%) for the cattle lairage module is shown in

Table **19**. These outputs only considered infected consignments within each scenario, and the categories defined were:

- Non-infected animals from non-infected herds (the same as the on-farm output)
- Infected herds:
    - Main lot:
        - Animals with no MAP infection (non-infected and no lesions, focal, multifocal) and low levels of external MAP
        - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
        - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
    - Separated lot (during antemortem inspection activities):
        - Animals with low level of MAP infection (Paucibacillary) and high level of external MAP
        - Animals with high level of MAP infection (Multibacillary) and high level of external MAP
        - Condemned animals

Within infected consignments, most animals (>99%) in all scenarios, have no MAP internally (non-infected animals and animals with no lesion, focal or multifocal lesions) and low MAP externally due to faecal cross-contamination from other animals in the consignment. Similarly to sheep, most of the carcasses and product will originate from animals with low level of MAP on the carcass surface and only a very low to negligible proportion of animals would have internal infection and high level of MAP externally.

Separation of clinically affected animals is very unlikely and therefore, the proportion of animals being separated is *extremely low* to *negligible*. This is similar for the condemned animals. As such, most animals with low to high level of internal infection will enter the slaughter floor and be used for human consumption, posing a risk for human exposure.

Within the main lots, there is a higher probability of animals with low and high MAP internal and external in consignments of adult dairy cattle (S3, S4 and S5) than in consignments of young beef cattle (S1 and S2), as the within-herd prevalence used in this assessment was higher for dairy than for beef cattle herds and more adult dairy cows were considered to be within the paucibacillary and multibacillary disease pathology category than young beef cattle.

Table 19. Predicted probability of cattle within various categories of MAP infection, within infected flocks, enter the slaughter line from the lairage. Predictions are presented by scenario* and consignment type† and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

	Probability	
Scenario / Consignment type	**Among animals from infected herds**	
	Median	5% - 95%
S1 Beef / S2 Beef		
Main lot		
No MAP internal / Low MAP External	0.9997	(0.9991 – 0.9999)
Low MAP internal / High MAP external	5.0×10^{-5}	(1.0×10^{-5} – 0.0002)
High MAP	0.0002	(5.0×10^{-5} – 0.0007)
Separated lot		
Low MAP internal / High MAP external	4.0×10^{-8}	(1.9×10^{-9} – 2.8×10^{-7})
High MAP	2.2×10^{-6}	(1.8×10^{-7} – 9.9×10^{-6})
Condemned	4.2×10^{-7}	(3.2×10^{-8} – 2.4×10^{-6})
S3 Dairy / S4 Dairy / S5 Dairy		
Main lot		
No MAP internal / Low MAP External	0.9959	(0.9932 – 0.9977)
Low MAP internal / High MAP external	0.0007	(0.0003 – 0.0017)
High MAP	0.0032	(0.0018 – 0.0054)
Separated lot		
Low MAP internal / High MAP external	6.3×10^{-7}	(3.7×10^{-8} – 3.5×10^{-6})
High MAP	3.2×10^{-5}	(4.4×10^{-6} – 8.9×10^{-5})
Condemned	6.2×10^{-6}	(7.3×10^{-7} – 2.2×10^{-5})

S1, Beef, Protected and Free zones; S2, Beef, Beef Protected and Management areas; S3, Dairy, Beef Protected area; S4, Dairy, Management area; S5, Dairy, Protected zone); † Separated lot = Lot of animals clinically affected with Johne's disease, detected and separated during antemortem inspections at lairage.

4.2.3 Abattoir

Table 20 shows the predicted amount of MAP (Log10 CFU) in a serving size of product for an average animal of each infected consignment. The average amount of MAP in product among animals from beef herds (S1 and S2) is very similar as the proportion of animals within each of the disease category as described in the lairage module is very similar within infected consignments. The same is reported among dairy consignments (S3, S4 and S5).

Among main lots from infected flocks (lots with non infected, no lesions, focal and multifocal lesions, paucibacillary and multibacillary animals), the amount of MAP in prime cut from beef cattle coming from S1 and S2, is estimated to be -0.02 (-0.63 – 0.46) Log CFU/100 g. Skeletal muscle from dairy cattle (S3, S4 and S5) has a higher estimated amount of MAP

(0.16; -0.56 – 2.10 Log CFU/100g). Liver produced from beef cattle (S1 and S2), has a median amount of MAP of 0 (0 - 0) Log CFU/100 g, indicating that this product poses a negligible risk of MAP exposure to humans. Intestines from adult dairy cattle (S3 and S4), have a median amount of MAP per 10 g of product of 0 Log CFU, since most animals in an infected consignment will not be infected and will only have MAP on the carcass surface; however, the 95 percentile of the output distribution is 4.70 Log CFU, representing the infected animals.

Table 20. Predicted amount of MAP (Log10 CFU) in a serving size of product for an average animal within an infected consignment. Predictions are presented by scenario*, consignment type‡ and animal product, and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

Scenario / Consignment type	**Total MAP Log CFU in a serving size† of product**		
	Median	5%	95%
S1 Beef			
Main lot Prime cut	-0.02	-0.63	0.46
Separated Prime cut	3.51	3.33	3.65
S2 Beef			
Main lot Prime cut	-0.02	-0.62	0.48
Separated Prime cut	3.51	3.32	3.66
Main lot Liver	0.00	0.00	0.00
Separated lot Liver	3.82	3.64	3.94
S3 Dairy			
Main lot Skeletal Muscle	0.16	-0.56	2.10
Separated Skeletal Muscle	3.55	3.39	3.68
Main lot Intestines	0.00	0.00	4.70
Separated Intestines	6.23	5.45	6.68
S4 Dairy			
Main lot Skeletal Muscle	0.16	-0.54	2.10
Separated Skeletal Muscle	3.55	3.39	3.68
Main lot Intestines	0.00	0.00	4.73
Separated Intestines	6.22	5.45	6.68
S5 Dairy			
Main lot Skeletal Muscle	0.16	-0.56	2.10
Separated Skeletal Muscle	3.55	3.39	3.39

S1, Beef, Protected and Free zones; S2, Beef, Beef Protected and Management areas; S3, Dairy, Beef Protected area; S4, Dairy, Management area; S5, Dairy, Protected zone); †Serving size: 100 g muscle and liver, 10g of intestines;
‡ Separated lot = Lot of animals clinically affected with Johne's disease, detected and separated during antemortem inspections at lairage.

Those consignments coming from separated animals during antemortem inspection entering the slaughter line, which are infected animals with low and high level of infection (Paucibacillary and Multibacillary animals only), have the highest amount of MAP in final product. For example, prime cut from beef cattle from S1 and S2 have an estimated amount of MAP of 3.51 (3.33 – 3.66) Log CFU / 100 g. A very similar estimate is obtained for skeletal muscle from separated lots from dairy cattle. The estimated median amount of MAP in intestines is 6.23 (5.45 – 6.68) Log CFU/ 10 g. However, as shown in the lairage module, the probability of these animals entering the slaughter line in a separated lot is *extremely low* to *negligible*, and therefore, most of these animals will enter the slaughter line with the main lot.

4.2.4 Overall probability of exposure

Table 21 presents the overall probability of animals with the different disease pathology categories entering the slaughter line among all animals coming from each scenario (infected and non-infected flocks), considering the entire production chain from the farm to the product. Most animals entering the slaughter line (>99%) from S1, S2 and S5, will be non-infected animals from non-infected flocks, posing a negligible risk of MAP exposure to humans. All other categories among animals from these scenarios are *very low* to *negligible* to occur.

A lower proportion of dairy cattle from S3 (88.4%; 86-8% - 89.9%) and S4 (79.8%; 78.9% - 80.7%) would come from non-infected herds as the herd prevalence in these areas is higher than in S1, S2 and S5. Approximately 20% and 12% of the rest of the animals from S3 and S4, respectively, would be non-infected animals (No MAP internal) with low MAP on the carcass surface. An *extremely low* proportion of S3 and S4 animals would be infected (low or high MAP internal) with high MAP externally.

Table 21. Predicted probability of cattle within different MAP infection categories enter the slaughter line. Predictions are presented by scenario* and consignment type, and are given as median, 5th and 95th percentiles of the output probability derived from 5,000 iterations of a simulation stochastic model.

	Probability	
Geographical area / Consignment type	**Among all animals from each scenario**	
	Median	5% - 95%
S1 Beef		
No MAP - Non Infected herds	0.9999	(0.9999 – 0.9999)
No MAP internal / Low MAP external	2.5×10^{-5}	(1.8×10^{-6} – 0.0001)
Low MAP internal / High MAP external	1.1×10^{-9}	(5.5×10^{-11} – 7.8×10^{-9})
High MAP	5.5×10^{-9}	(2.7×10^{-10} – 3.7×10^{-8})
No food chain	8.9×10^{-12}	(2.6×10^{-13} – 1.0×10^{-10})
S2 Beef		
No MAP - Non Infected herds	0.9989	(0.9987 – 0.9992)
No MAP internal / Low MAP external	0.0010	(0.0008 – 0.0012)
Low MAP internal / High MAP external	5.2×10^{-8}	(9.8×10^{-9} – 1.6×10^{-7})
High MAP	2.5×10^{-7}	(4.8×10^{-8} – 7.6×10^{-7})
No food chain	4.3×10^{-10}	(3.2×10^{-11} – 2.4×10^{-9})
S3 Dairy		
No MAP - Non Infected herds	0.8839	(0.8680 – 0.8987)
No MAP internal / Low MAP external	0.1156	(0.1008 – 0.1314)
Low MAP internal / High MAP external	8.6×10^{-5}	(2.9×10^{-5} – 0.0002)
High MAP	0.0004	(0.0002 – 0.0006)
No food chain	7.2×10^{-7}	(8.4×10^{-8} – 2.6×10^{-6})
S4 Dairy		
No MAP - Non Infected herds	0.7981	(0.7887 – 0.8073)
No MAP internal / Low MAP external	0.2010	(0.1919 – 0.2104)
Low MAP internal / High MAP external	0.0002	(5.1×10^{-5} – 0.0004)
High MAP	0.0007	(0.0004 – 0.0011)
No food chain	1.3×10^{-6}	(1.5×10^{-7} – 4.5×10^{-6})
S5 Dairy		
No MAP - Non Infected herds	0.9989	(0.9955 – 0.9999)
No MAP internal / Low MAP external	0.0010	(7.6×10^{-5} – 0.0044)
Low MAP internal / High MAP external	7.2×10^{-7}	(4.7×10^{-5} – 4.2×10^{-6})
High MAP	3.2×10^{-6}	(2.3×10^{-7} – 1.6×10^{-5})
No food chain	5.4×10^{-9}	(2.4×10^{-10} – 4.6×10^{-8})

S1, Beef, Protected and Free zones; S2, Beef, Beef Protected and Management areas; S3, Dairy, Beef Protected area; S4, Dairy, Management area; S5, Dairy, Protected zone);

4.2.5 Sensitivity analysis

The influence of herd prevalence (P_H), within-herd prevalence (P_U) probability of multibacillary animals showing clinical signs (*Prob_CS*), probability of sending multibacillary animals to the abattoir (related to the disease pathology node of the on-farm scenario) and the probability of antemortem detection (*ProbAM*), on the probability of infected animals with high level of MAP being entering the slaughter line from S4 is shown in Figure 8. As expected, the input value with more influence on the output of the model evaluated is the herd prevalence. When 10% of the herds from S4 are infected, the probability of animals with high level of MAP entering the slaughter line would decrease by 2-fold. If the prevalence increases to 40% and 80%, this output probability increases by 2 and 4-fold respectively. Similarly, the effect of within-herd prevalence is also significant, with a 3-fold decrease on the output probability when the prevalence is 1%, and a 3-fold increase with the prevalence increases to 10%. This indicates that having accurate estimates of both herd and within-herd prevalence is important to provide an accurate estimate of the probability of MAP exposure to humans.

Similar effect than for the sheep model is seen regarding the probability of sending multibacillary animals to the abattoir. When this probability decreases to 0.2, the probability of animals with high MAP infection entering the slaughter line decreases approximately 3-fold. The effect of the probability of multibacillary animals showing clinical signs and the antemortem inspection activities detecting and separating clinically affected animals is very limited.

The influence of the within-herd prevalence (P_U), probability of multibacillary animals showing clinical signs (*Prob_CS*), probability of sending multibacillary animals to the abattoir (related to the disease pathology node of the on-farm scenario), the probability of antemortem detection (*ProbAM*) and the amount of grams of faeces on the carcass, on the amount of MAP in final product (*Skeletal muscle from S4 animals*) was also investigated and results are shown in

Figure **9**. The within-herd prevalence has a significant influence on the amount of MAP on the final product, which could increase by 5-fold when the within-prevalence increases to 10%, supporting the need for a better understanding of the within-herd prevalence for dairy and beef cattle in Australia. Increasing the *ProbAM* would decrease the amount of MAP in product from animals of the main lot, as most multibacillary animals would be separated. However, this would be dependent on the animals showing clinical signs. Similar than for the

sheep model, the most significant effect on this output is due to a variation of the grams of faeces in the carcass, as expected, indicating the importance of maintaining good hygienic practices at the abattoir to reduce carcass contamination with faeces.

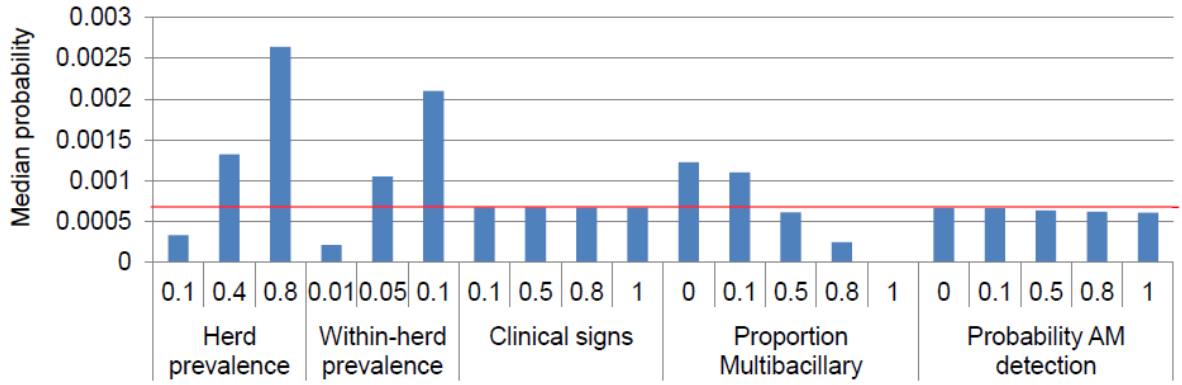

Figure 8. Sensitivity analysis depicting the influence of key input variables on the median probability (solid horizontal line) of infected animals with high level of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) entering the slaughter line at the abattoir. Key input variables evaluated: a) herd level prevalence of Johne's disease, b) within-herd prevalence of Johne's disease, c) probability of infected animals showing clinical signs, d) b) probability of multibacillary-diseased animals being sent for slaughter and e) probability of detection of MAP clinical cases at ante-mortem inspection. Height of bars represents the median probability of infected animals with high level of MAP entering the slaughter line at the abattoir according to each value used for the specific input variable. Results were obtained from a simulation of 5,000 iterations using @Risk's Advanced Sensitivity Analysis.

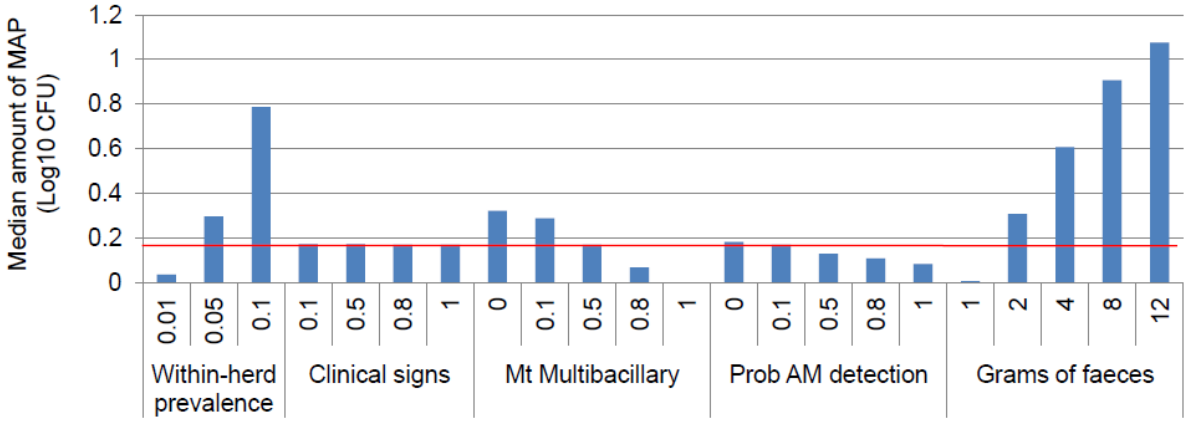

Figure 9. Sensitivity analysis depicting the influence of key input variables on the median amount (solid horizontal line) of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) CFU (Log10) in 100g of skeletal muscle of an adult dairy cattle from an infected consignment originating from Scenario 4 (high herd prevalence) considered in this assessment. Key input variables evaluated: a) within-herd prevalence of Johne's disease, b) probability of infected animals showing clinical signs, c) probability of multibacillary-diseased animals being detected on farm and not sent to the abattoir, d) probability of detection of MAP clinical cases at ante-mortem inspection and e) Grams of faeces present on the carcass surface. Height of bars represents the median amount of MAP according to each value used for the specific input variable. Results were obtained from a simulation of 5,000 iterations using @Risk's Advanced Sensitivity Analysis.

4.3 Identification of information gaps

During this exposure assessment, the following information gaps were identified:

- For cattle:
    a. Information on herd prevalence and within herd-prevalence for beef cattle and dairy herds.
    b. Proportion of infected animals within each pathology category, in herds with different level of disease prevalence.
    c. Proportion of clinical cattle that die on farm or are not sent for slaughter.
    d. Performance of Silirum®, novel killed vaccine against Johne's disease in cattle, in Australian beef herds and dairy herds particularly in relation to proportion of infected animals within each pathology category and level of faecal shedding by infected animals within each pathology category.
    e. MAP level and distribution of disseminated infection in infected animals and specifically for the four product categories (prime cut, intestines, liver and skeletal muscle).
- For sheep:
    a. More accurate information on flock prevalence and within-flock prevalence
    b. MAP level and distribution of disseminated infection in infected animals and specifically for the four product categories (prime cut, intestines, liver and skeletal muscle).
    c. Information about proportions of flocks vaccinated in different geographic and prevalence areas.
    d. Proportion of animals of different age groups vaccinated in different geographic and prevalence areas.
    e. Proportion of clinical sheep that die on farm or are not sent for slaughter.
- Transportation (sheep and cattle)
    a. Level of cross-contamination and transfer of MAP between animals during transportation from farm direct to abattoir.
- Saleyards (sheep and cattle)
    a. The geographic range of source farms for animals at major saleyards in Australia and composition by prevalence area of consignments bought at saleyards and taken direct to abattoirs for slaughter.
    b. Management of clinical Johne's disease animals at saleyards and any removal of clinical animals for welfare or other concerns that occurs, and

would thus reduce the number of heavily infected animals included in saleyard consignments taken direct to abattoir for slaughter.

c. Level of cross-contamination and transfer of MAP between animals during period held at saleyards and then transported from saleyard to abattoir.

- Lairage (sheep and cattle):
    a. Probability of detection and separation of Johne's disease clinically affected animals by the stockman and antemortem inspection.
    b. Probability of separated animals being condemned antemortem.
    c. Mixing cattle from different origins at lairage.
- Slaughter line:
    a. Implementation of Good Hygienic Practices and assumptions regarding its impact on MAP level.
    b. Grams of faeces present in carcasses at the end of the slaughter line.

4.4 Identification of potential mitigation strategies:

During the process of developing the exposure pathways and conducting the sensitivity analysis, some critical control points along the chain of the production of meat products, and potential mitigation strategies that could limit the risk of MAP exposure to humans were identified.

According to the sensitivity analysis the most important aspects to consider as potential mitigation strategies or measures to limit the human exposure to MAP are:

- *Flock/herd and within flock/herd prevalence:* According to the model outputs and the sensitivity analysis, flock/herd infection status is the most significant factor on the probability of infected animals entering the slaughter line and as such posing a risk for human exposure to MAP. Therefore, any measure with the aim of controlling disease at on-farm level (e.g. vaccination, biosecurity plans) will be considered as a potential mitigation strategy. In areas with high prevalence (GA2 and S4 in the current assessments) it is important to manage the level of disease, with the aim of reducing the flock/herd prevalence. In those areas with low prevalence (such as GA3), it is crucial that this level of prevalence is not increased (as shown in the sheep sensitivity analysis, Section 4.1.5), maintaining biosecurity and management practices to avoid the introduction and spread of disease.

- *Increased awareness among producers about the implications of sending infected animals with multibacillary level of infection to the abattoir.* For both, sheep and cattle, the models indicate that increasing the probability of detecting multibacillary animals on the farm would decrease the probability of infected animals being sent to the abattoir; however, the probability of detecting multibacillary animals on the farm is dependent on the animals showing clinical signs. For sheep, the probability of infected animals showing clinical signs is limited, as such, the effect of the probability of detection of multibacillary animal on the farm is limited. For cattle, this influence of this parameter might be more significant as more infected cattle than sheep would show clinical signs (Cousins et al., 2002).

- *Identification of Johne's disease clinically infected animals at lairage:* If Johne's disease clinically infected animals, which are those shedding higher quantity of MAP in faeces, are separated from the rest of the lot and slaughtered at the end of the kill day, potential cross-contamination would be reduced. However, the sensitivity

analysis indicates that this input parameter has minimal effect on the output of the models, as detection is dependent on the animals showing clinical signs.

- *Grams of faeces on carcass:* The sensitivity analyses indicate that among infected consignments, the amount of faeces present in the carcass surface has a very significant effect on the amount of MAP in product. This supports the importance of maintaining good hygienic practices at the abattoir to reduce potential faecal contamination of the carcass from the hide and the intestines. This input value was estimated using expert opinion, and as such, the level of uncertainty about this estimate might have been significant.

Other potential mitigation strategies not investigated by the sensitivity analysis were also identified during the process of the exposure assessment:

- *Identification of infected flocks/herds at lairage:* If Johne's disease status of the batch of animals sent to the abattoir is known, risk-based management could be applied to reduce the potential risk of cross-contamination of non-infected animals. Lots originating from known infected flocks/herds could be slaughtered at the end of the kill day. Johne's disease herd or flock status information could be elicited on the National Vendor Declaration and this then linked to the National Livestock Identification Scheme (NLIS).
- *Requirement of all sheep being crutched before being sent to the abattoir:* This would reduce the presence of faecal material and as such, the risk of cross-contamination of non-infected animals
- *Removal of lymph nodes:* This would reduce the level of MAP in the final product, especially in muscle and liver.
- *Segregation or condemnation of carcasses if gross lesions are identified:* This would reduce the risk of exposure to humans; however, we would need to consider sensitivity and specificity of identification of Johne's disease by gross pathology.

5 Discussion

The current study assessed the probability of carcasses from Johne's disease infected animals (sheep and cattle) and animals contaminated with MAP being produced for human consumption and estimated the amount of MAP present in different animal products at the end of the slaughter line. This exposure assessment has followed the OIE methodology for risk assessment (OIE, 2009) using a modular process risk model approach to develop the pathways of exposure. With this approach, the red meat chain production process (for sheep and cattle) has been divided into different processing steps, which allowed for the identification and investigation of the stages with more influence on the probability of exposure of humans to MAP.

The exposure pathways and scenario trees have been developed following an extensive consultation process with the Project Steering Group and a combination of published literature, industry reports, unpublished studies and expert opinion has been used as data sources to populate the models used. These assessments have focused on specific scenarios for sheep and cattle, mainly based on Johne's disease prevalence areas and animal type, and only direct consignments from these areas have been considered. As such, the probability estimates calculated represent animals from these specific scenarios and originating from direct consignments from the farm.

We acknowledge that consignments originating from saleyards can account for a significant proportion of animals arriving at an abattoir; however, the main factor affecting the potential exposure to humans is the flock/herd infection status which depends on the geographic origin of the consignment and requires ability to configure saleyard consignments by geographic area for farms of origin. Development of a module that represented the complex situation at saleyards, with animals entering from a variety of source farms, with farm numbers and geographic distributions that change over time, and with exits in consignments that go to farms and to abattoirs that vary in size and destination over time, was beyond the scope of this project. It is highly likely that some saleyard consignments arriving at abattoirs include Johne's disease infected animals, and for saleyards that draw stock from high prevalence areas it is probable that the proportion of animals with low and high MAP level of infection may even be higher than in direct consignment cohorts due to farmers moving low productive animals off farm via sale at saleyards. However, data to support the configuration of saleyard consignments going to abattoir for slaughter by MAP status was not available, and consideration of cross-contamination from shedding animals was also not possible.

Similarly, there were substantial information gaps that prohibited inclusion of a transport module in this assessment. When shedding animals are present in a direct consignment,

cross-contamination will occur during transportation from farm to abattoir increasing numbers of animals with hide contamination present on arrival at lairage. However the modelling required to estimate cross-contamination during transport was also beyond the scope of this project and substantial knowledge gaps exist in relation to parameters for such models. The lairage module has accounted for this cross-contamination to occur, with a change on the proportion of animals with different MAP external contamination; however, the magnitude of this cross-contamination is not well understood.

As previously mentioned, several information gaps have been identified during this study, and some input parameters used have significant uncertainty. Some of these parameters are the animal level prevalence in infected flocks/herds, the definition of different disease pathology categories in cattle, the amount of MAP in product originating from animals with different disease pathology categories and the grams of faeces present in carcasses at the end of the slaughter line. In the current assessments, uncertainty has been incorporated into the quantitative models using probability distributions. As such, accuracy of the results obtained relies on the accuracy of the input values used in the models and further research in some areas might reduce this uncertainty in the future.

When data were not available, formal expert opinion elicitation was used. Conjoint analysis and Delphi methods are the most common methods involving expert opinion that have previously been used in the field of animal health and risk analysis. Conjoint analysis, which does not involve group interaction, has been used in animal health studies to rank the risk of different farm-level risk factors for disease in livestock (Horst et al., 1996; Stark et al., 2002). However, uncertainty of the expert opinion is not considered when using this method. The Delphi method consists of rounds of questionnaire-based surveys among a group of experts. After each round, summaries of the group's responses are distributed among experts, who are asked to review their answers of the questionnaire according to the summaries provided. Expert opinion is usually elicited in the form of subjective confidence intervals, which provide information on the uncertainty related to the estimate; however, these interval judgments are highly susceptible to overconfidence (Speirs-Bridge et al., 2010). As Vose (2008) indicates the uncertainty in subjective estimates represents the inherent randomness of the variable and the uncertainty from the expert's lack of knowledge of the parameters describe that variability. To reduce overconfidence, Speirs-Bridge et al. (2010) suggest a 4-step interval elicitation process, which has been used in the current assessment. During this process, experts are asked to provide their confidence (50% to 100%) that the true value of the estimate will fall within their interval (minimum and maximum). When the expert confidence is very low in their first interval estimate, the derived intervals (80%) are extremely wide and with very low precision. This method suggests that if experts are presented with the results

of the wide derived intervals, they would not accept the lack of precision and would provide a higher confidence for their interval. Moreover, experts might modify their estimates after topics have been discussed among the group participants, according to the estimates of an individual in the group whose opinion is highly valued (Vose, 2008). However, the revision of the experts' estimates after showing them the responses of the group often does not provide responses closer to the correct answer (Vose, 2008).

Potential biases and errors when eliciting expert opinion, previously described by Vose (2008), that could have a significant impact on the validity of the risk analysis model, are: 1) The ability of the expert to remember past occurrences of similar events; 2) The representativeness of the experts involved with the elicitation process; 3) The 'anchoring' to the most likely value and estimation of the minimum and maximum by adjustment from the most likely value estimated (source of overconfidence); 4. Inaccuracy of estimates due to: Inexpert expert, conflicting agendas, unwillingness to consider extremes, eagerness to say the right thing, units used in the estimation, expert too busy and belief that the expert should be quite certain.

The results suggest that it is possible for highly infectious (multibacillary) animals to be sent to slaughter even after accounting for loss of animals due to death on farm and exclusion of animals due to being unfit for transport and slaughter. The possibility of multibacillary animals to be sent to slaughter is higher from the high prevalence geographic area. Adult sheep sent to slaughter are more likely to be highly infectious than 1 year old lambs because the proportion of multibacillary animals is much higher among adult sheep than young 1 year old sheep. Similarly, dairy cattle sent to slaughter are more likely to have a high level of MAP infection than beef cattle, as the herd prevalence and within-herd prevalence are higher among dairy than beef cattle herds, and the proportion of multibacillary animals within adult dairy cows is higher than among beef cattle younger than 2 years of age.

Although it is possible for infected animals to be sent to slaughter, the probability is very low to extremely low. Even from a high prevalence geographic region, the probability of multibacillary animals to be sent to slaughter is only 5 per 10,000 adult sheep and even lower for lambs (2 per 10,000 lambs). Among dairy cattle in the higher prevalence areas, the probability of multibacillary animals being sent to slaughter is only 4 to 7 per 10,000 adult dairy cows. These probabilities are negligible among beef cattle. The reason for this is that most animals from this region would be non-infected animals from non-infected flocks, and among the rest, most animals (>98%) would be non-infected.

When the entire production chain form the farm to the product is considered, most animals entering the slaughter line are estimated to be non-infected from non-infected properties, especially in areas of low Johne's disease prevalence (GA1 and GA3 for sheep, S1, S2 and

S5 for cattle). These animals do not pose a risk to human exposure to MAP. In addition, among the rest of the animals most would be non-infected animals with low levels of MAP on the carcass surface due to cross-contamination in lairage, and an extremely low to negligible proportion of animals would be infected with high MAP contamination on the carcass surface. Among adult sheep from GA2, 6 per 10,000 animals would have a low level of internal MAP infection and high level of MAP contamination on the carcass surface, and 4 per 10,000 animals would have high levels of MAP internally and externally. Among dairy cattle from the scenario with highest prevalence (S4), 2 per 10,000 animals would have a low level of internal MAP infection and high level of MAP contamination on the carcass surface; and, and 7 per 10,000 animals would have high levels of MAP internally and externally. This indicates that although the probabilities of infected animals entering the slaughter line are extremely low, it is possible that highly infected animals enter the slaughter line. In addition, we acknowledge that cattle herd-prevalence as well as sheep animal-level prevalence might be underestimated. Therefore, the probabilities of exposure reported by these models may also be somewhat underestimated.

The results suggest that it is possible for MAP to be present in red meat products from a low proportion of direct consignments. In these products it is estimated that the amount of MAP can be as high as about 9 logs CFU in 10 g of intestines and about 5.5 log CFU in 100 g of skeletal muscles from adult infected sheep from separated consignments during antemortem inspection activities (only infected animals in the consignments). However, the median amount of MAP is likely to be lower (0.45 to 1.24 log CFU per 100 g in skeletal muscles from animals from the main lots (infected and non-infected animals). Similar results are observed in cattle, with amount of MAP as high as 6.2 log CFU in 10 g of intestines and 3.5 log CFU in 100g of skeletal muscle from adult dairy cows from separated consignments during antemortem inspection activities (only infected animals in the consignments). The median amount of MAP is estimated to be lower (-0.02 to 0.16 log CFU per 100 g) in skeletal muscles from animals from the main lots (infected and non-infected animals). As such, it is possible that an animal with high level of MAP in product ends up in the food chain. An example would be a subclinical old dairy cow, in which case the amount of MAP in product would be high, thus posing an unacceptable risk for human exposure. The levels of product contamination reported by this model are likely to be detectable with current culture tests (R. Whittington, personal communication).

Disease prevalence has a significant influence on the probability of sending animals infected with high level of MAP to the abattoir, as well as the amount of MAP in product. This supports the importance of having accurate estimates of flock/herd and the animal level prevalence. In addition, this indicates that maintaining biosecurity and using good

management practices to avoid the spread of disease between properties and from geographical areas with high prevalence is very important for controlling the risk of MAP exposure to humans.

In sheep, vaccination against Ovine Johne's disease was found to be a major factor influencing the level of MAP in the end product. In sensitivity analyses, increasing flock vaccination to 100% among infected flocks caused a 3 fold reduction and reducing it to 10% caused a 3 fold increase in the probability of high MAP animals entering the slaughter line. This suggests that maintaining high vaccination coverage among infected flocks is important for reducing the probability of exposure of MAP to humans. However, the effect of increasing the proportion of animals vaccinated within a vaccinated flock on the final product was minimal. Even when all young sheep are vaccinated the probability of animals with high MAP infection entering the slaughter line only decreases from a median of 5 to 4 per 10 000, suggesting that there would be very limited effect on vaccinating young animals to be sent to the abattoir at this age. The main explanation for this limited effect is that the proportion of multibacillary animals within young sheep is very low independent of vaccination status.

At this time, no vaccine for Bovine Johne's disease is commercially available to cattle producers in Australia. Silirum®, a novel killed vaccine against Johne's disease in cattle, has been trialled in infected dairy herds in Victoria since 2005 under a restricted permit however the results are not yet available. From research in other countries, it is anticipated that Silirum® when administered to young animals in infected herds will reduce level of faecal shedding and extent of pathology and clinical disease. If these effects are achieved by vaccination of infected cattle herds in Australia then, similar to Gudair vaccination of infected sheep flocks, a reduction in probability of high MAP cattle entering the slaughter line would be expected.

Variation of the grams of faeces in the carcass also had a major impact on the amount of MAP in the product, indicating the importance of maintaining good hygienic practices at the abattoir to reduce carcass contamination with faeces. In addition, this input value had significant uncertainty as it was estimated through expert opinion. More accurate information on the actual amount of faeces on the carcass surface at the end of the slaughter line is required to reduce this uncertainty.

Other factors influencing the probability of MAP exposure to humans is the age of slaughtered sheep and the probability of not sending multibacillary animals to the abattoir. Regarding age of slaughtered sheep, only slaughtering young sheep could be used as a strategy in the future to provide low MAP or MAP free meat to highly conscious local or international buyers. If only 20% of the multibacillary animals are sent to slaughter instead of 40 to 70% estimated in this assessment (due to increased farmer awareness), the probability

of sending high MAP animals would decrease by half; suggesting that awareness and attitudes of producers regarding sending animals with Johne's disease clinical signs are important in reducing the risk of MAP exposure to humans. However, only a proportion of multibacillary animals show clinical signs, therefore the effect of this input parameter would actually be limited. Similarly, the effect of antemortem inspection activities in detecting and separating clinically affected animals is very limited as only those animals showing clinical signs (approximately 1% and 10% of paucibacillary and multibacillary animals, respectively) are able to be detected and separated. In addition, since the clinical signs of Johne's disease are not very specific, with several other common conditions showing similar clinical signs, such as nutritional deficiencies and nematodiasis, any separation method based on clinical sings would likely to incur significant costs due to false positive animals being discarded from the food chain.

Results from this study, provide an insight into the risk of exposure of humans to MAP posed by the cattle, dairy and sheep industries in Australia, and identify which geographical areas and production systems pose a comparatively higher risk. Results suggest that the risk posed by the red meat chain in Australia is low as most animals entering the slaughter line destined for human consumption originate from non-infected properties. However, these results also suggest that the risk is not negligible, and that measures to reduce the flock and herd prevalence as well as the animal level prevalence, and to maintain good hygienic practices at the abattoirs to avoid or reduce carcass contamination are crucial to control and manage this risk. This study has also highlighted the need for further research in some areas, such as prevalence, disease pathology, amount of MAP in product and carcass contamination, for a better quantification of the risk of MAP exposure to humans.

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8 Appendix

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Appendix 2 – Expert elicitation – Sheep flock vaccination

Information about a Meat and Livestock Australia Project to assess the likelihood of exposure of humans to MAP through the consumption of red meat

Please complete the following table:

a) List the local government areas you service in the first column.

b) Indicate the percentage of flocks vaccinated for Ovine Johne's disease (regardless of their infection status) in the next three columns.

c) Specify in the last shaded column how confident are you (from 50 to 100%) that the true percentage will fall within the minimum-maximum range you have given.

Num	Your Local government areas	Minimum %	Most likely %	Maximum %	Confidence %
1					
2					
3					
4					
5					
6					

Your name and contact details (optional)

Thank you for providing this information. It would help us in conducting the exposure assessment.

Please contact **Dr Marta Hernandez-Jover**, the principal investigator of this project if you need any information about this project:

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Appendix 3 – Expert elicitation – Disease pathology in sheep and cattle

Sheep

Question: Assume that there are 100 Ovine Johne's disease infected young (<2 Year old) and 100 Ovine Johne's disease infected adult sheep (>2 year old) in a flock.

- Of these how many do you think would have multibacillary, paucibacillary and Perez 1 and 2 scores? Please specify what would be most likely, minimum and maximum numbers of sheep in these categories.
- State how confident you are (from 50 to 100%) that the true numbers will fall within the minimum-maximum range?

	<2 Year old (n=100)			>2 year old (n=100)		
	Perez 1 or 2	Paucibacillary	Multibacillary	Perez 1 or 2	Paucibacillary	Multibacillary
Most likely						
Minimum						
Maximum						
Confidence						

Question: Among the multibacillary animals in this flock, which proportion would not be sent to the abattoir, due to animal mortality and/or not being fit for transport?

Cattle

Question: Gonzalez et al. (2005) conducted a study using 167 cows between 1.5 and 7 years old, and reported 79.0% of cows with no lesion, focal and or multifocal lesions, 19.2% of cows with multibacillary/intermediate lesions and 1.8% of cows with paucibacillary lesions. What is your opinion regarding the accuracy of these proportions?

Question: What would be the proportion of infected young cattle (< 2 years old) within the first category (no lesions/local/multifocal)?

Minimum %	Most likely %	Maximum %	Confidence %

Question: Among the multibacillary animals in this herd, which proportion would not be sent to the abattoir, due to animal mortality and/or not being fit for transport?

Appendix 4 – Expert elicitation – Antemortem inspection and pre-slaughter practices and activities at the abattoir

FOR CATTLE ABATTOIR

Request a description of the cattle abattoir – daily kill, proportion of herd types, adult and calves, major markets and products

ANTE-MORTEM INSPECTION

Considering the current practices at this abattoir, in a scenario where cattle are presented at the abattoir with clinical signs of Johne's disease, then:

1. What do you abattoir personnel conducting antemortem inspection recognize as clinical signs of Johne's disease in cattle?
2. Routinely – what % of times would animals showing these clinical signs be separated from the lot in the lairage?

   Do you think this would vary? Is there a range around this?

   - Most likely value
   - Minimum value
   - Maximum value
3. When such animals are separated, what would be the reason for separating them?
4. What % of these separated animals would be condemned and not used for human consumption?

PRE-SLAUGHTER WASH

1. Is there a pre-slaughter wash facility at the abattoir?
2. Is the pre-slaughter wash facility in use? Y/N If Yes – please describe the washing facility and routine process implemented for washing cattle pre-slaughter
3. Describe an animal that will be selected for pre-slaughter wash.
4. What are the criteria at this abattoir for designation of animal/s for pre-slaughter wash?
5. If one or a few animals in a consignment meet these criteria will the affected animals only be washed or will all the consignment be washed?
6. At this abattoir, from 100 lines of cattle processed how many lines would undergo a pre-slaughter wash?

   - Most likely value

- Minimum value
- Maximum value

7. Are there Any Other Measures taken to reduce contamination up to start of kill line or just after stunning?
8. Does mixing of consignments occur in lairage? What size and cattle type of consignments are typically mixed?

FOR SHEEP ABATTOIR

Request a description of the sheep abattoir – daily kill, proportion of direct consignment and from saleyard, adult and lambs, major markets and products

ANTE-MORTEM INSPECTION

Considering the current practices at the abattoir you are working on, in a scenario where SHEEP are presented at the abattoir with clinical signs of Johne's disease, then:

1. What do you consider abattoir personnel conducting antemortem inspection recognize as clinical signs of Johne's disease?
2. Routinely – what % of times would animals showing these clinical signs be separated from the lot in the lairage?

   Do you think this would vary? Is there a range around this?

   - Most likely value
   - Minimum value
   - Maximum value

3. When such animals are separated, what would be the reason for separating them?
4. What % of these separated animals would be condemned and not used for human consumption?

PRE-SLAUGHTER MANAGEMENT

1. Is there a pre-slaughter wash facility at the abattoir?
2. Is the pre-slaughter wash facility in use? Y/N If Yes – please describe the washing facility and routine process implemented for washing sheep pre-slaughter
3. Are there Any Other Measures taken to reduce contamination up to start of kill line or just after stunning?

4. Does mixing of consignments occur in lairage? What size and types (direct consignment, saleyard) of consignments are typically mixed?

Appendix 5 – Expert elicitation – Grams of faeces on the carcase surface at the end of the slaughter line

Exposure Assessment for *Mycobacterium avium* subspecies *paratuberculosis* (MAP)

PROJECT NO. A.MFS.0273

Expert elicitation - Grams of faeces on the carcase surface at the end of the slaughter line

The amount of faeces present on the carcase surface could be an indicator of the effectiveness of the good hygienic practices applied at the abattoir; however, measuring the actual grams of faeces on the carcase is virtually impossible and monitoring programs focus on measuring the level of Total Viable Counts (TVC) and the presence or absence of *E. coli*, coliforms and *Salmonella* spp.

The presence of generic E. coli on carcases is specific for faecal contamination, however, in many instances counts are "nil". Although TVC is not specific for faecal contamination it has the advantage of being inevitably present on carcases and thus counts area a good descriptor of variation in processing performance. The following table presents the TVC data (Log10 / g of carcase) collected through the ESAM program in 2012 for different classes of livestock and different abattoirs.

	Sheep	Lamb	Cow & Bull	Steer/Heifer
Abattoirs (n)	22	22	37	41
Mean	2.61	2.46	2.76	2.66
Standard Deviation	3.95	3.64	4.00	4.22
Minimum	0	0	0	0
Maximum	5.80	5.48	5.73	6.23
Minimum mean (among all abattoirs)	0.77	1.36	0	0.30
Maximum mean (among all abattoirs)	3.62	3.36	3.89	4.22

We would appreciate if you could answer the questions below individually and bring your answers tomorrow. Responses of all participants will be anonymously collated and discussed among participants during the day. Following discussion, you will be asked to answer the same questions a second time. The second round of responses will be those used for the study and will not be shared among participants.

Thanks in advance for your collaboration in this research project.

Dr. Marta Hernandez-Jover

Project principal investigator

Question 1:
For animals processed in abattoirs in Australia, how many grams of faeces will be present on the TOTAL carcase surface for the following scenarios:

Carcass from:	***Abattoirs with the LOWEST mean TVC /g of carcass***		
	Most likely	Minimum	Maximum
Sheep			
Lamb			
Cow & Bull			
Steer/Heifer			

Carcass from:	***Abattoirs with the HIGHEST mean TVC /g of carcass***		
	Most likely	Minimum	Maximum
Sheep			
Lamb			
Cow & Bull			
Steer/Heifer			

How confident are you (from 50 to 100%) that the true values will fall within the minimum-maximum ranges? _____

Question 2

Regarding the mass of faeces deposited onto the carcase surface, fill in the following table in relation to the proportion (as a %) of total faecal mass that is derived from hide as opposed to derived from gut?

	Most likely %	Minimum %	Maximum %
% from hide			

How confident are you (from 50 to 100%) that the true values will fall within the minimum-maximum ranges? _____

Question 3:

Do you have any qualifying remarks to make about your estimates above or the general issue of faecal contamination on carcases?